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Chemical Preparation Laboratory for IND Candidate Compounds

Final Report

E.M. Schubert, Ph.D.

August 10, 1990

(January 17, 1985 - January 16, 1990)

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

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Pharm-Eco Laboratories, Inc.
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REPORT DOCUMENTATION PAGE

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17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
07	03		RA I, BD, Compounds, ~~IND~~ Candidates Nucleosides, Alkaloids, Heterocycles, Triazoles, Antivirals
06	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

During the reporting period several classes of compounds were prepared on scales ranging from several grams to kilogram quantities. These classes of IND candidate compounds can be subdivided into modified nucleosides, alkaloids with synthetically modified precursors and analogues, and miscellaneous heterocycles that possess biological activities. All compounds were submitted for antiviral testing.

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I. Summary

During the reporting period 55 submissions have been sent to the contracting agency, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland. The delivered compounds had been synthesized by the contractor according to published procedures while strictly following current Good Manufacturing Practice (CGMP). In addition exploratory research was executed for scale-up preparations to allow for the bulk synthesis of IND - compounds as potential antiviral agents.

The compounds submitted for testing can be subdivided into three structural classes: modified nucleosides, alkaloid-type natural products, and miscellaneous heterocyclic aromatics and aliphatics. The complexities of the syntheses ranged from one-step procedures to 18-step intricate structural manipulations to yield from grams up to multi-kilo batches of final products.

II. Foreword

All information in this report is the property of the U.S. Army Medical Research and Development Command. The contractor retains no copyright or patent rights.

All target compounds reported herein were prepared in strict compliance with "Current Good Manufacturing Procedures" (CGMP) guidelines. All intermediates and final products unreported in the chemical literature were fully characterized by elemental and spectral analyses.

III. Cumulative list of compounds completed and delivered to U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) January 17, 1985 to January 16, 1990.

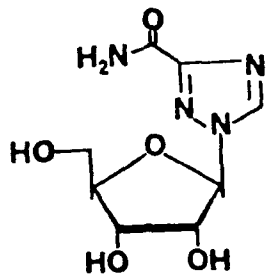
I.D. NO.	COMPOUND	AMOUNT	SYN. REFERENCE * (ANN. CONT. RPT.)
AVS 1	1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide(Ribavirin)	2.0 Kg 571 g	1985, p. 17 1986, p. 12
AVS 52	3-ß-D-ribofuranosyl-1(H)pyrazolo[4,3-d]-pyrimidin-7(6H)thione (Thioformycin-B)	10.2 g 5.85 g	1985, p. 6 1989, p. 7
AVS 79	9-ß-D-ribofuranosyl-purine-6-thiocarboxamide	20.0 g 14.5 g	1985, p. 8 1988, p. 45
AVS 94	4-Hydroxy-1,2,3-triazole-5-carboxamide	12.5 g	1988, p. 39
AVS 206	1-ß-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride	28.0 g 70.0 g 410.4 g 1948 g	1986, p. 7 1987, p. 8 1987, p. 10 1988, p. 41
AVS 206 AE	1-ß-D-Ribofuranosyl-1,2,4-triazole-3-methylamidate	1.15 g	
AVS 215	3-Deazaguanosine	24.0 g	1987, p. 29
AVS 222	3-Bromo-4-chloropyrazolo[3,4-d]pyrimidine	63.1 g	1985, p. 12
AVS 253	2-ß-D-ribofuranosylselenazo-4-carboxamide	37.8 g 36.8 g	1985, p. 13 1986, p. 25
AVS 253 TA	2',3',5'-Tri-O-acetyl-2-ß-D-ribofuranosyl-selenazo-4-carboxamide	13.0 g	1986, p. 28
AVS 266	4-Iminopyrazolo[3,4-d]-1,3-thiazine-6(7H)thione	15.4 g	1986, p. 13
AVS 272	3-Deazaguanine	25.3 g	1987, p. 29
AVS 332	Methyl-4-chloro-5-(2,4-dichlorophenyl)--1 (H)-pyrazole-3-carboxylate	25.0 g	1986, p. 9
AVS 353	Combretastatin	9.5 g	1987, p. 20
AVS 353 AC	Combretastatin acetate	1.0 g	1987, p. 23
AVS 360	Lycoricidine Triacetate	4.6 g 7.1 g	1986, p.19 1988, p. 14
AVS 360 DH	4aH-r,4H-trans,2H-cis,10bH-trans, 1,2-Dihydroxy-8,9-methylenedioxy-1,2-4a,10b-tetrahydro-6(5H)phenanthridone	2.5 g	1986, p.31

I.D. NO.	COMPOUND	AMOUNT	SYN. REFERENCE * (ANN. CONT. RPT.)
AVS 360 HP	4H-r,1H-trans,2H-cis,10bH-trans-1-(2',Tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)phenanthridone	6.4 g	1986, p. 30
AVS 360 MA	1-Hydroxy-2-acetyl-lycoricidine	4.0 g	1987, p. 44
AVS 360 MA	4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1,3,4-Trihydroxy-2 acetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone	1.23 g	1988, p. 16
AVS 360 OH	4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,-1-Hydroxy,2,3,4-triacetoxy-8,9-methylenedioxy-1,2,3,4,-4a,10b-hexahydro-6(5H)phenanthridone	450 mg	1987, p. 38
AVS 360 TA	4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans,1,2,3,4-Tetraacetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone	1.84 g	1987, p. 61
AVS 360 TH	4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1,2,3,4-Tetrahydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone	4.4 g 1.5 g	1987, p. 49 1987, p. 55
AVS 439	7-Carbamoyl-1-ß-D-ribofuranosylimidazo-[1,2-b]pyrazol-6-yl-methyl sulfone	12.0 g	1986, p. 14
AVS 593	cis,trans 3,6-diethoxy-tetrazadiphosphorine-3,6-disulfide	10.15 g	1985, p. 16
AVS 4071	1-ß-*D*-Ribofuranosyl-1,2,4-triazole-3-methylimidale	21.0 g	1988, p. 23
AVS 4600	1-(2.3-Dideoxy-ß-*D*-glycero-pent-2-enofuranosyl)1,2,4-triazole-3-carboxamide	1.5 g 2.8 g	1988, p. 30 1988, p. 30
AVS 4601	2',3'-Dideoxyribavirin	1.1 g 2.3 g	1988, p. 31 1988, p. 31
AVS 4602	3'-Deoxyribavirin	1.1 g	1988, p. 28
AVS 4603	1-(2,3'-Anhydro-ß-*D*-ribofuranosyl)-1,2,4-triazole-3-carboxamide	1.6 g	1988, p. 27
AVS 4604	2',3'-Dideoxytiazofurin	1.7 g	1988, p. 33
AVS 4605	2-(5'-Hydroxymethylfuran-2-yl)		

I.D. NO.	COMPOUND	AMOUNT	SYN. REFERENCE * (ANN. CONT. RPT.)
AVS 4606	2',3'-Dideoxy-2',3'-didehydro-tiazofurin	1.2 g	1988, p. 35
AVS 5058	1-ß-D-Ribofuranosyl-1,2,4-triazole-N-methyl-carboximidate hydrochloride	15.1 g	1989, p. 13
ARA ADA	9-(2',3'-Di-*O*-acetyl-ß-*D*-arabinofuranosyl)adenine	23.0 g 25.5 g	1987, p. 18 1987, p. 18
AVS DAU	3-Deazauridine	45.0 g	1986, p. 11
AVS INT	4aH-r,1H-trans,1H-Hydroxy-1,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone	220 mg	1987, p. 34
AVS INT II	1-Hydroxy-8,9-methylene dioxy-1,4,4a, 106-tetrahydro-6(5H)-phenanthridone	2.08 g	1988, p. 22
AVS OCT	4-nitro-3-(octanoyloxy) benzoic acid	12.0 g	1989, p. 14
AVS RCOOH	1-ß-*D*-Ribofuranosyl-1,2,4-triazole-3-carboxylic acid	4.7 g	1988, p. 12
AVS TAE	Methyl-1,2,4-triazole-3-carboxylate	5.0 g	1989, p. 12
AVS TCA	1,2,4-triazole-3-carboxamide	4.0 g	1988, p. 11
AVS TCOOH	1,2,4-triazole-3-carboxylic acid	4.0 g	1988, p. 10
AVS TFN	Thiazofurin nitrile	380 mg	1987, p. 32

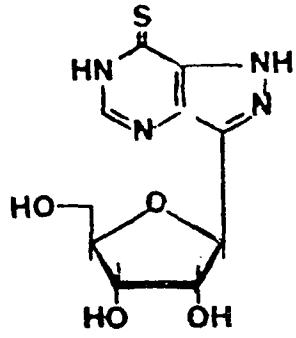
*) SYN. REFERENCE (ANN. CONT. RPT.) = Synthesis Reference: See Annual Contract Report.

IV. Structures of Compounds Submitted



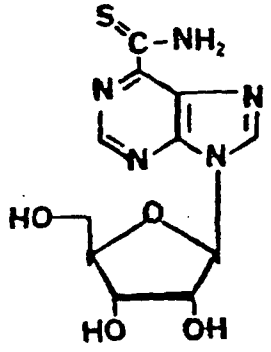
1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide(Ribavirin)

AVS 1



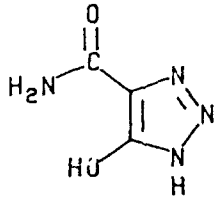
3-ß-D-ribofuranosyl-1(H)pyrazolo[4,3-d]-pyrimidin-7(6H)thione (Thioformycin-B)

AVS 52



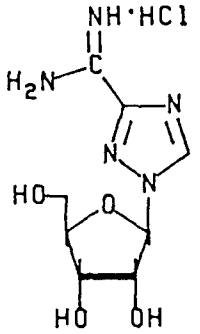
9-ß-D-ribofuranosyl-purine-6-thiocarboxamide

AVS 79



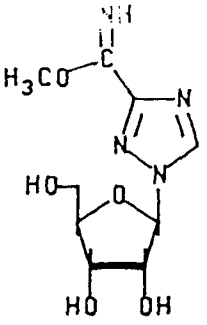
4-Hydroxy-1,2,3-triazole-5-carboxamide

AVS 94



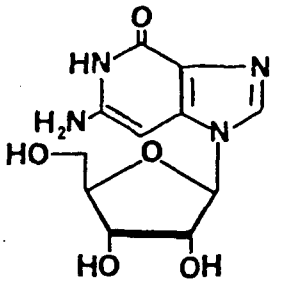
1-ß-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride

AVS 206



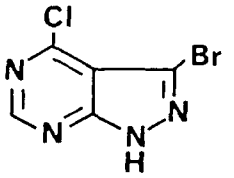
1-ß-*D*-Ribofuranosyl-1,2,4-triazole-3-methylamidate

AVS 206 AE



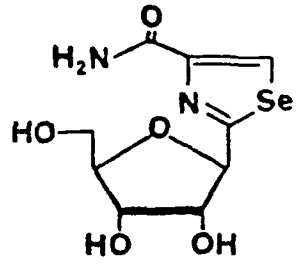
3-Deazaguanosine

AVS 215



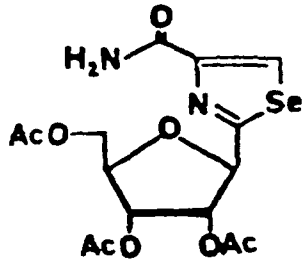
3-Bromo-4-chloropyrazolo[3,4-d]pyrimidine

AVS 222



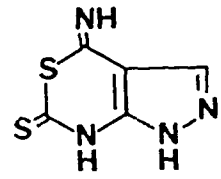
2-ß-D-ribofuranosylselenazo-4-carboxamide

AVS 253



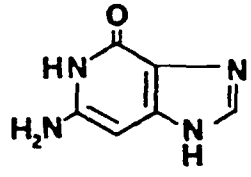
2',3',5'-Tri-O-acetyl-2-ß-D-ribo-furanosyl-selenazo-4-carboxamide

AVS 253 TA



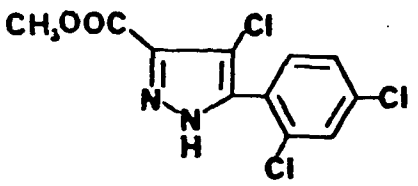
4-Iminopyrazolo[3,4-d]-1,3-thiazine-6(7H)thione

AVS 266



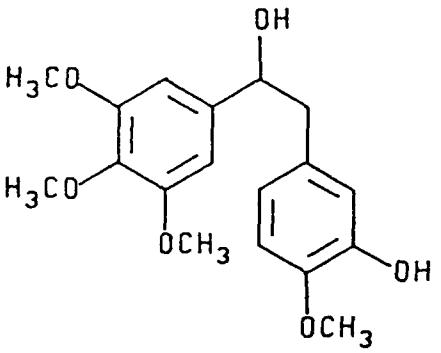
3-Deazaguanine

AVS 272



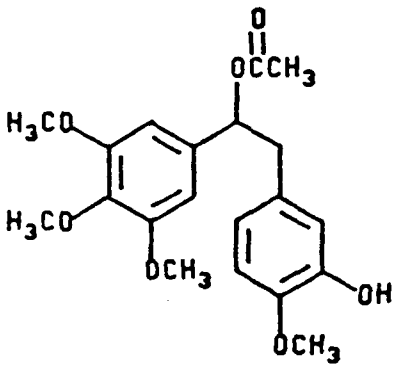
Methyl-4-chloro-5-(2,4-dichlorophenyl)-1 (H)-pyrazole-3-carboxylate

AVS 332



Combretastatin

AVS 353



Combretastatin acetate

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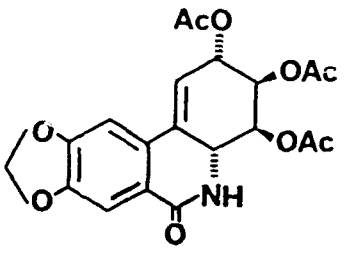
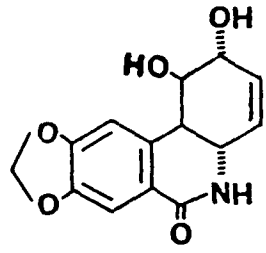


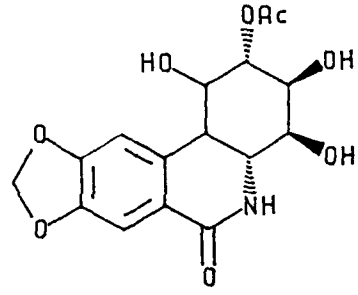
Lycoricidine Triacetate

AVS 360



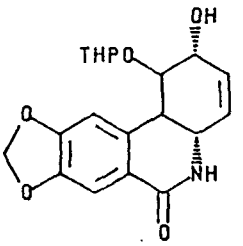
4aH-r,1H-trans,2H-cis,10bH-trans,1,2-Dihydroxy-8,9-methylenedioxy-1,2-4a,10b-tetrahydro-6(5H)phenanthridone

AVS 360 DH



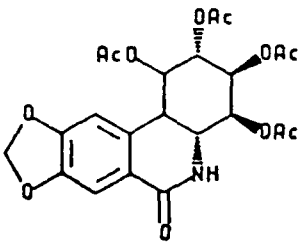
1-Hydroxy-2-acetyl-lycoricidine

AVS 360 MA



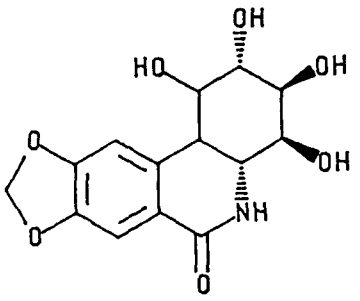
4H-r,1H-trans,2H-cis,10bH-trans-1-(2',Tetrahydropyranyloxy)-2-hydroxy- 8 , 9 - methylenedioxy-1,2,4a,10b-tetra-hydro-6(5H)phenanthridone

AVS 360 HP



4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans,1,2,3,4-Tetraacetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone

AVS 360 TA



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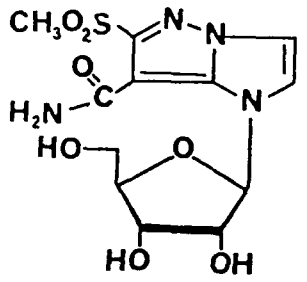
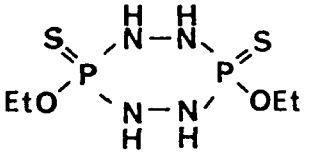
AVS 360 TH



7-Carbamoyl-1-ß-D-ribofuranosylimidazo-[1,2-b]pyrazol-6-yl-methylsulfone

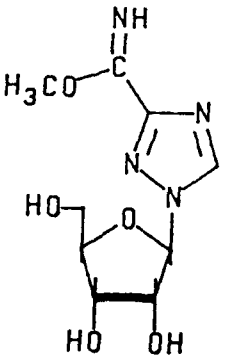
AVS 439



cis;trans

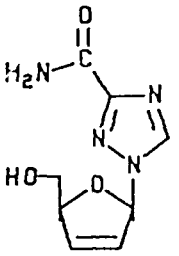
cis,trans 3,6-diethoxy-tetrazadiphosphorine-3,6-disulfide

AVS 593



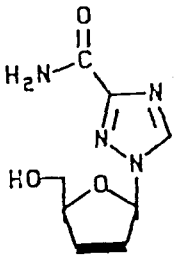
1-ß-*D*-Ribofuranosyl-1,2,4-triazole-3-methylimidale

AVS 4071



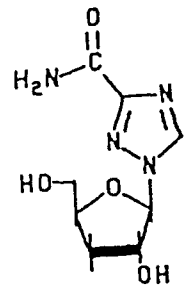
1-(2.3-Dideoxy-ß-*D*-glycero-pent-2-enofuranosyl)1,2,4-triazole-3-carboxamide

AVS 4600



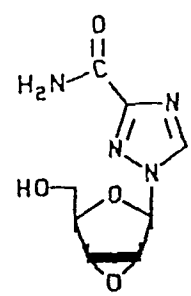
2',3'-Dideoxyribavirin

AVS 4601



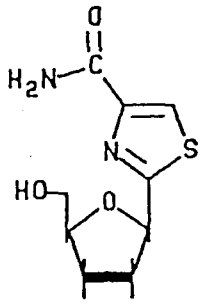
3'-Deoxyribavirin

AVS 4602



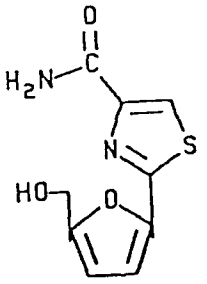
1-(2,3'-Anhydro-ß-*D*-ribofuranosyl)-1,2,4-triazole-3-carboxamide

AVS 4603



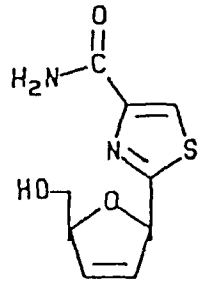
2',3'-Dideoxytiazofurin

AVS 4604



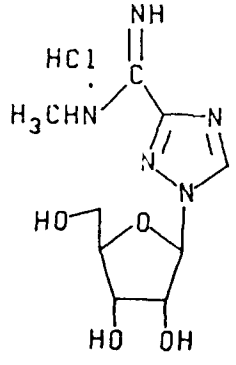
2-(5'-Hydroxymethylfuran-2-yl)thiazole-4-carboxamide

AVS 4605



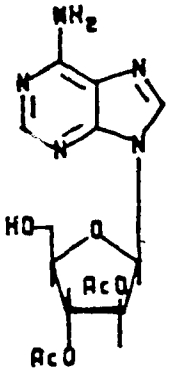
2',3'-Dideoxy-2',3'-didehydro-tiazofurin

AVS 4606



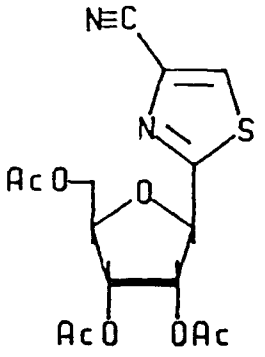
1-ß-D-Ribofuranosyl-1,2,4-triazole-N-methyl-carboximidate hydrochloride

AVS 5058



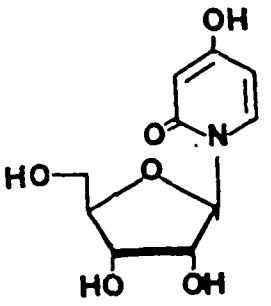
9-(2',3'-Di-*O*-acetyl-ß-*D*-arabinofuranosyl)adenine

ARA ADA



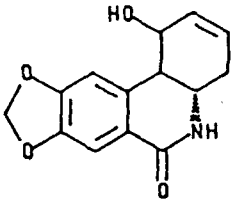
2(Tri-O-acetyl-ß-*D*-ribofuranosyl)thiazole-4-carboxamide

AVS ATF



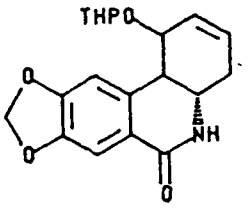
3-Deazauridine

AVS DAU



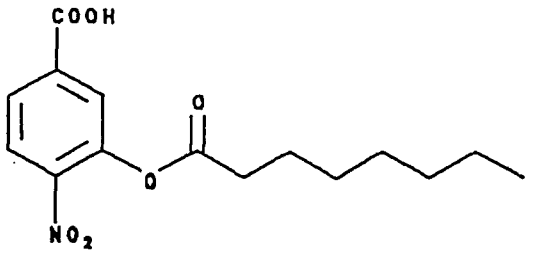
4aH-r,1H-trans,1H-Hydroxy-1,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone

AVS INT



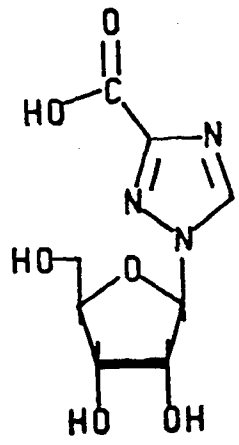
4aH-r,1H-trans,1-(2'-Tetrahydropyranloxy)-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)-phenanthridone

AVS INT 12



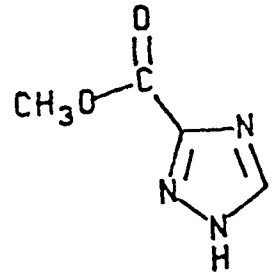
4-nitro-3-(octanoyloxy) benzoic acid

AVS OCT



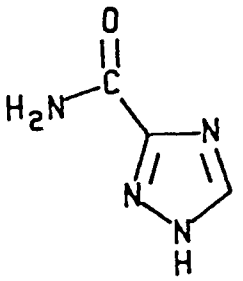
1-ß-*D*-Ribofuranosyl-1,2,4-triazole-3-carboxylic acid

AVS RCOOH



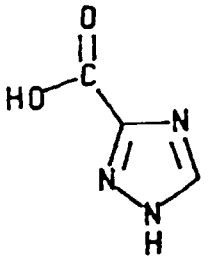
Methyl-1,2,4-triazole-3-carboxylate

AVS TAE



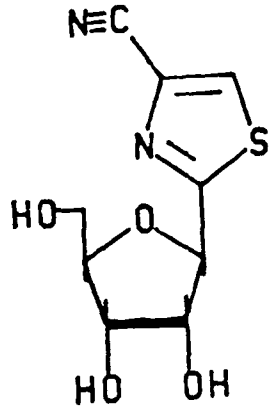
1,2,4-triazole-3-carboxamide

AVS TCA



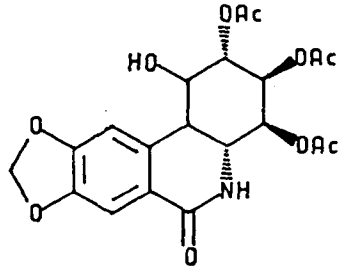
1,2,4-triazole-3-carboxylic acid

AVS TCOOH



Thiazofurin nitrile

AVS TFN



4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,1-Hydroxy,2,3,4-triacetoxy-8,
9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone

AVS 360 OH

V. Overview and Discussion

During the contract period from January 17, 1985 to January 16, 1990 a total of 55 submissions had been sent to the U.S. Army Medical Research Institute of Infectious Disease (USAMRIID). The majority of the compounds had been previously reported in the literature of exhibiting various degrees of antiviral activities. Such compounds were prepared on scales ranging from several grams to up to 2kg bulk quantities. The structures of several known antiviral or antitumor compounds were modified synthetically to possibly yield novel analogues that possess enhanced activities or show a specific mode of action. Based on their structural skeletons the submitted compounds fall into three groups of classes: modified nucleosides, natural products, and miscellaneous heterocycles.

A substantial amount of effort was allocated to large-scale preparation of 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin) (Scheme 1) Ribavirin with its documented antiviral activity was also the starting material to synthesize the carboxamidine analogue in greater quantities to enable large-scale in vivo studies in the treatment of Hemmorrhagic Fever with Renal Syndrome, where this modified derivative showed pronounced efficacy over Ribavirin.

Comparative structural studies between Ribavirin and Thiazofurin, a C-nucleoside of known antiviral activity, were performed to produce several deoxygenated species. Analoguous to active dideoxy purine nucleosides it was hoped that such synthetic manipulations would produce entities with greater antiviral activities, however, preliminary test results do not indicate such desirable effects. The chemical transformations leading to such deoxy-analogues of ribavirins and thiazofurin are shown in Schemes 2 and 3 respectively, and this study, submitted to the Journal of Nucleosides and Nucleotides, is presently in print.

In addition to preparing Ribavirin and structurally modifying commercially available nucleosides, several non-natural nucleoside were synthesized when starting with basic building blocks to produce the target compounds via a multi-step syntheses. Schemes 4, 5, and 6 show the preparations of 3-Deazaguanosine (AVS 215), 2-β-D-ribofuranosylselenazo-4-carboxamide (AVS 253, Selenazole) and 7-carbamoyl-1-β-D-ribofuranosylimidazo[1,2-b]pyrazol-6-yl-methyl-sulfone (AVS 439) respectively.

A significant amount of time and efforts was directed towards the synthesis of two natural products which had been previously isolated in minute quantities from plant extracts by researchers in Japan and the United States. Combretastatin (AVS 353), reported by G. Pettit, Arizona State University, was synthesized by the contractor according to Scheme 7, where the material was obtained as a racemic mixture. The second compound, Lycoricidine Triacetate (AVS 360), had been previously isolated and synthesized by Ohta et al., Kyote College of Pharmacy, following a 21-step synthetic pathway. The contractor was able to circumvent several manipulations and obtained the correct steric hydroxyl orientation as the key step, thus shortening the synthetic route to 17 steps, as shown in Scheme 8. The successful one-step cis-hydroxylation using osmium tetroxide presented a novel synthetic tool, and the study was submitted to and published in Synthesis, Aug. 1987.

A number of intermediates obtained during the lycoricidine synthesis, previously not available from natural sources and unreported in the literature, were submitted for antiviral screenings. Studies are still continuing with this class of compounds that have the phenanthridone skeleton in common, and a publication summarizing these investigations is the preparatory stage, to be submitted to the Journal of Medicinal Chemistry shortly.

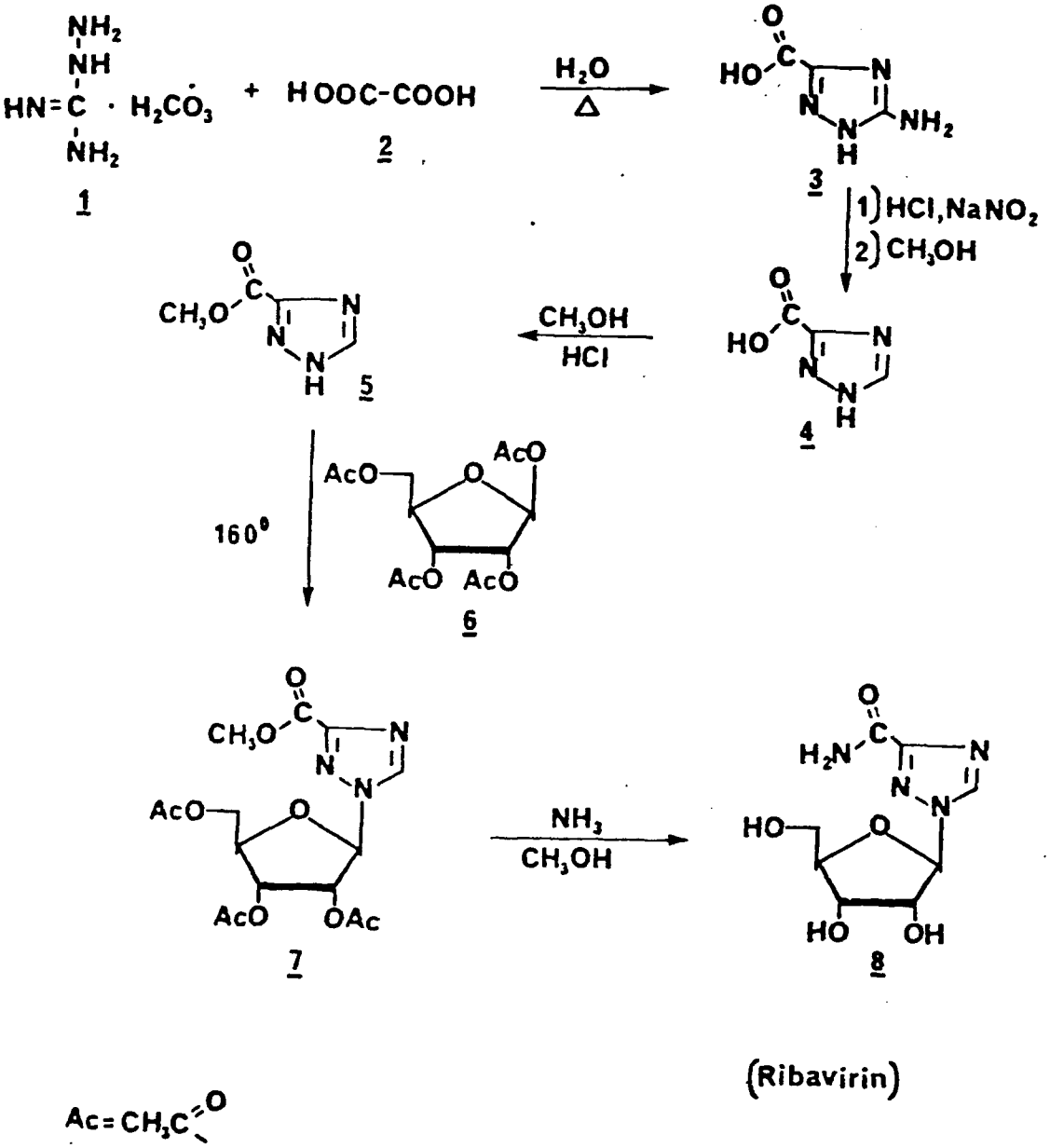
The heterocycles produced under this contract were mostly nucleobases, related to nucleosides that were prepared during the same period, however, their syntheses could differ greatly, as shown for 7-Deazaguanine (AVS 272) in Scheme 9. Non-nucleoside related heterocycles such as methyl-4-chloro-5-(2,4-dichlorophenyl)- 1(H) pyrazole-3-carboxylate (AVS 332) are an example of compounds that showed some biological activity, and its preparation is shown in Scheme 10.

The total scope of work encompassed all aspects of modern organic synthesis, combined with analytical evaluations based on current "State of the Art" techniques. Valuable chemical experience and special scientific insights the contractor gained through this work will always be greatly appreciated by the people involved in all aspects of contract work, and hopefully the contracting agency feels that the contractor's contributions helped to promote progress in their field of endeavour.

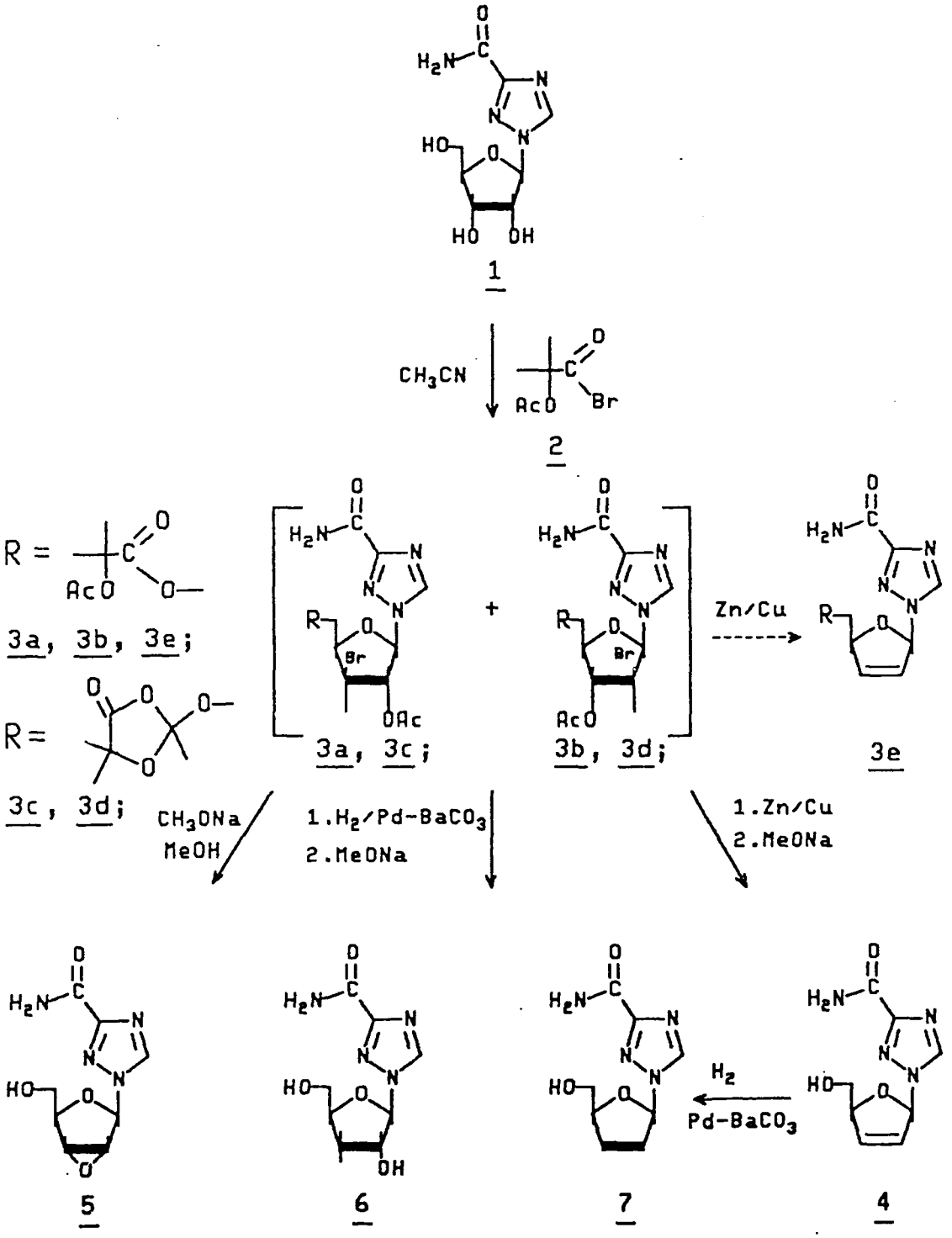
VI. Synthetic Reaction Schemes

Scheme 1

Synthetic Procedure Ribavirin

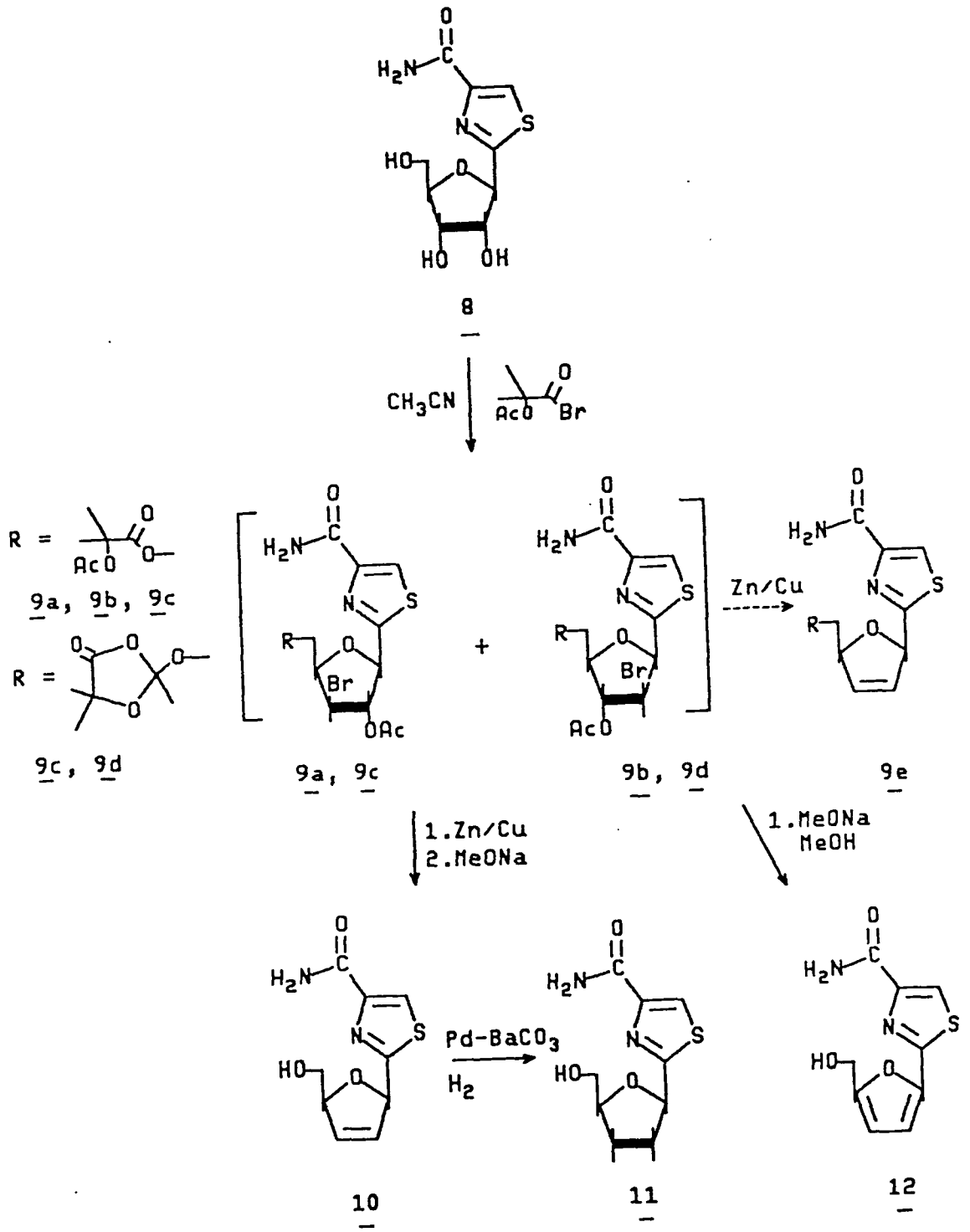


Scheme 2



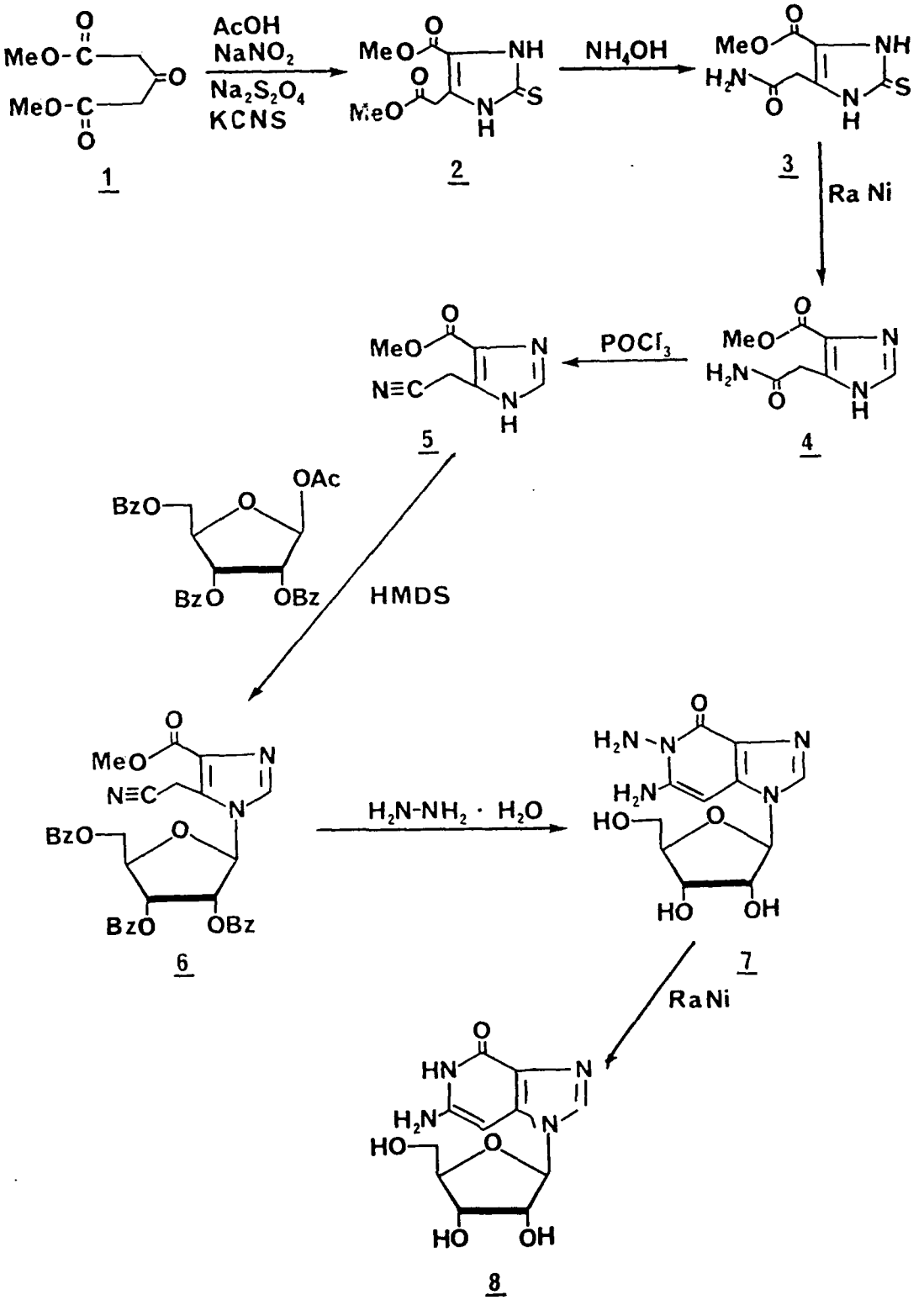
Ribavirin Series

Scheme 3



Tiazofurin Series

Scheme 4



1 2 3 4 5 6 7 8

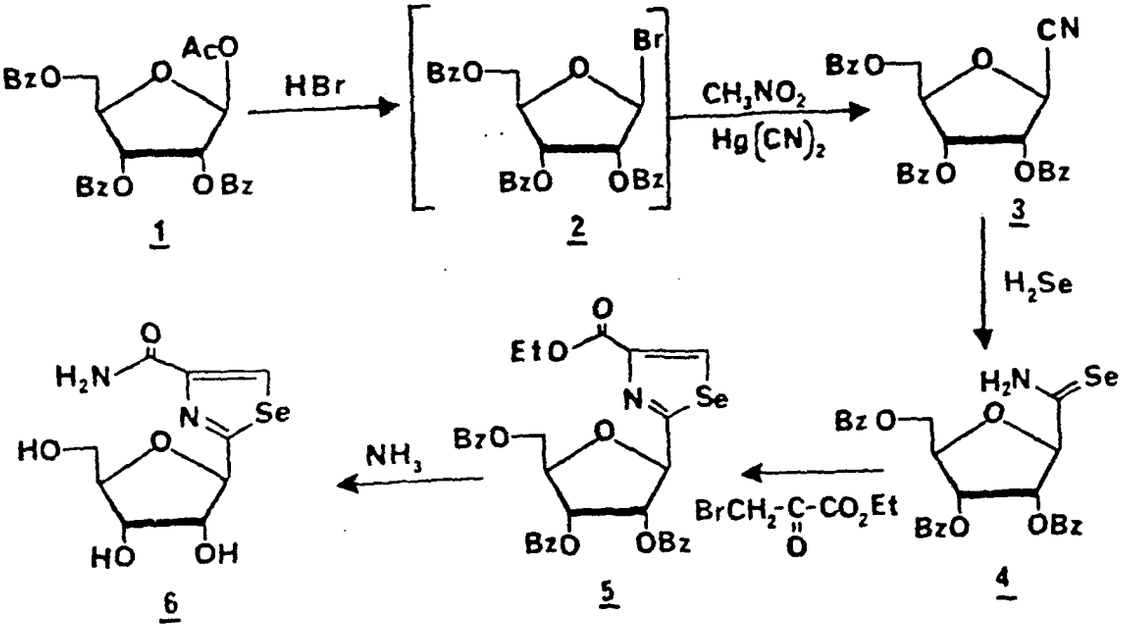
AVS 215

Scheme 5

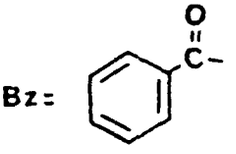
Synthetic Procedure

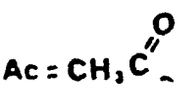
2-β-D-Ribofuranosylselenazo-4-carboxamide

AVS 253

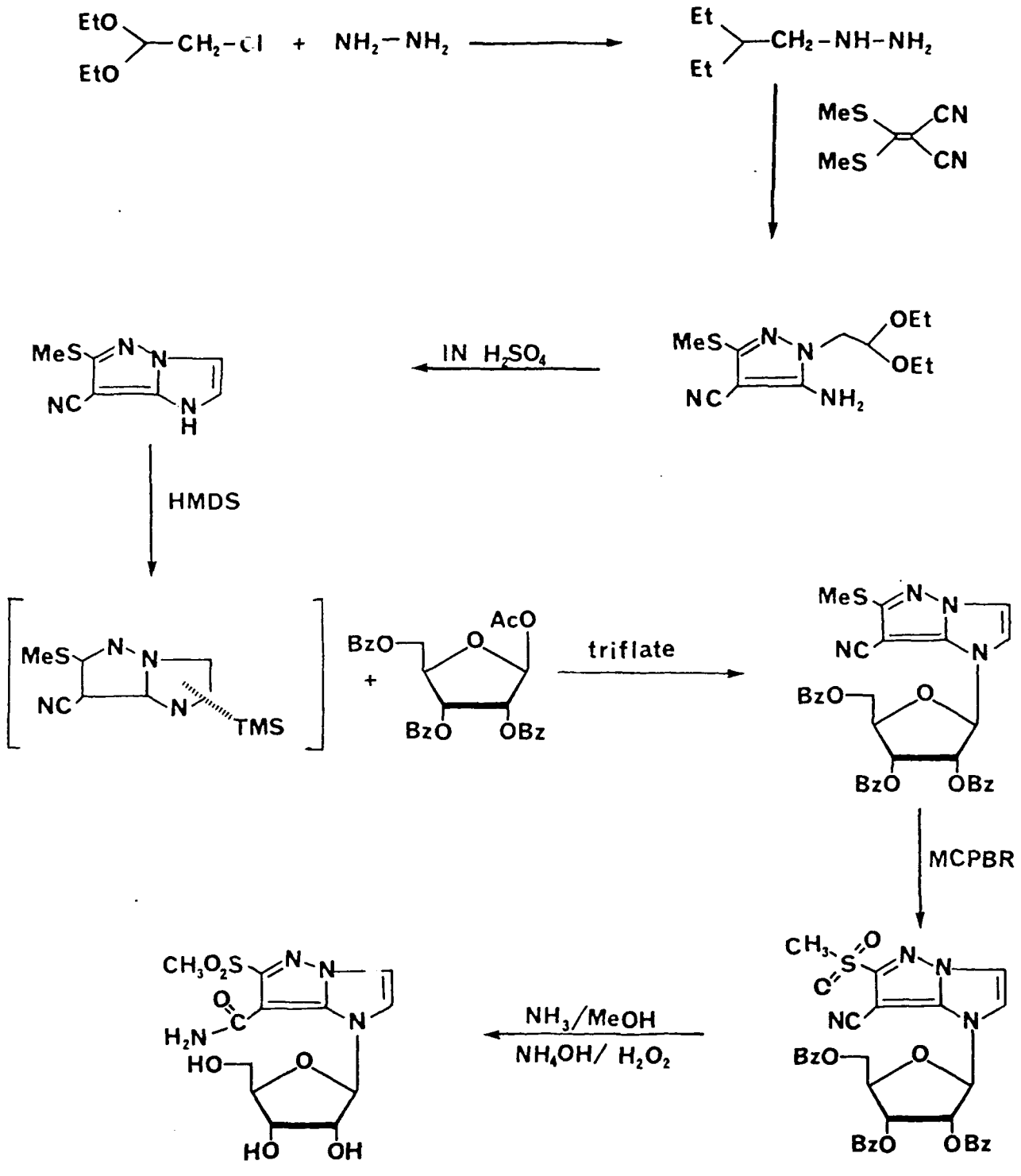


AVS 253



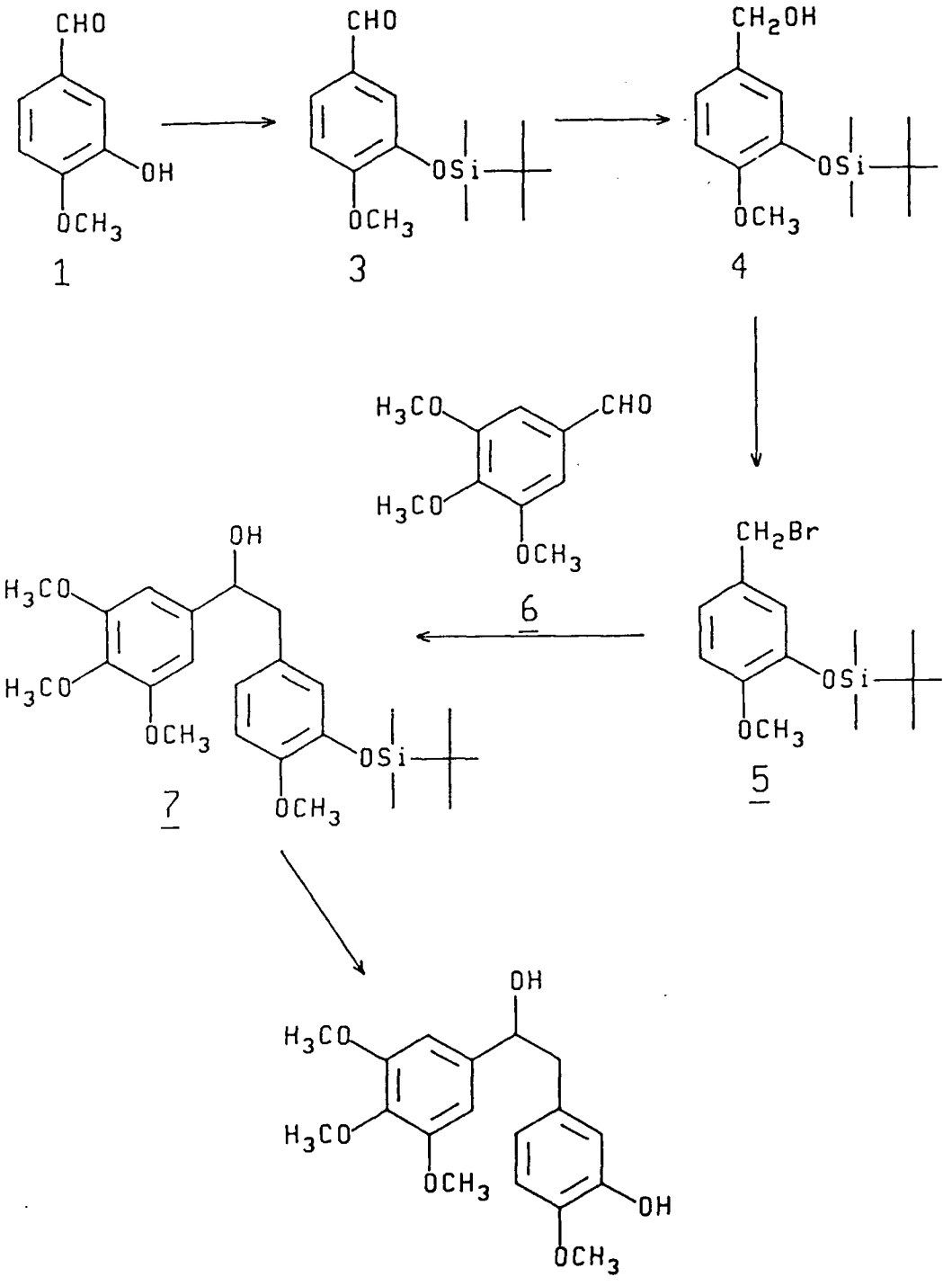


Scheme 6

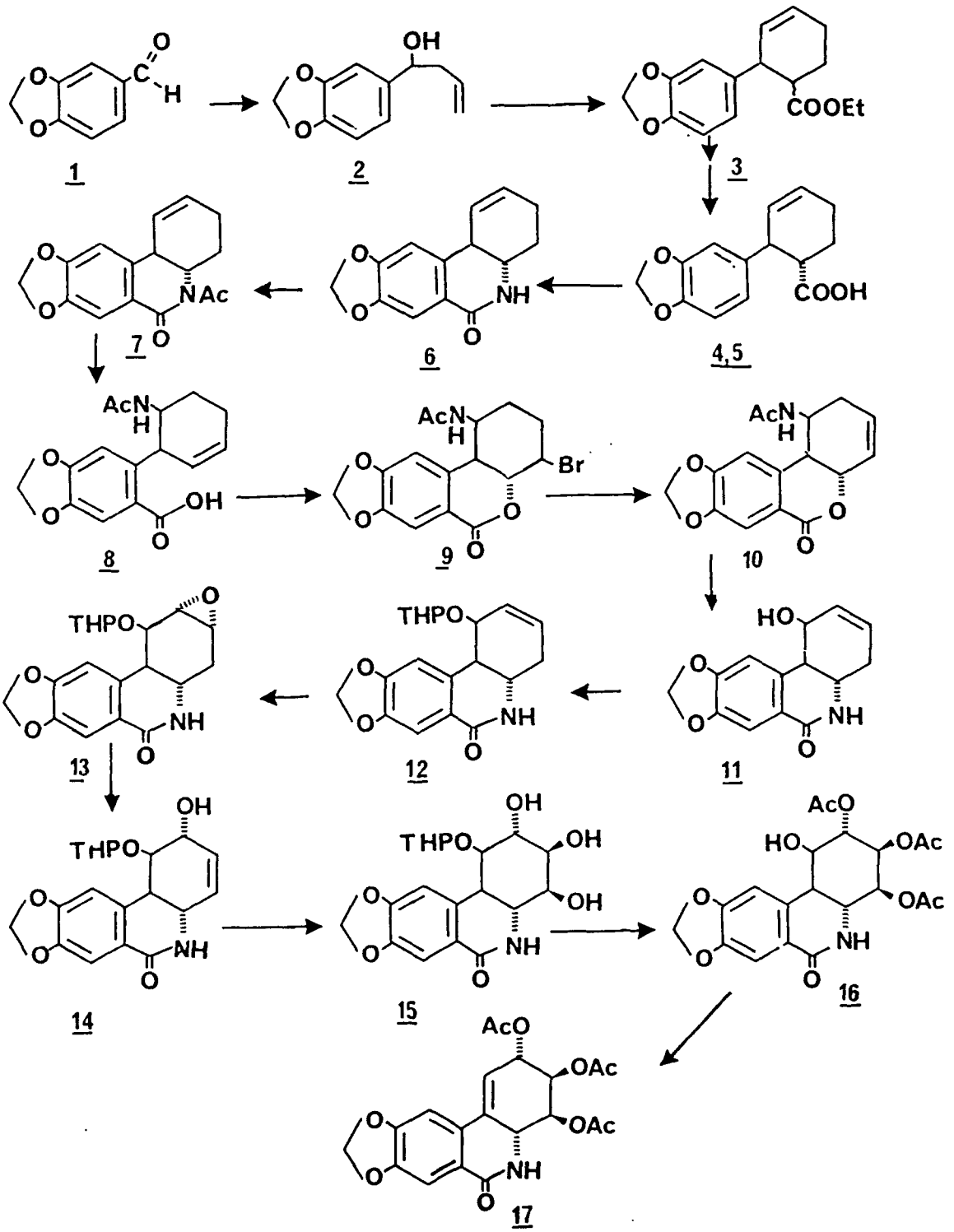


AVS 439

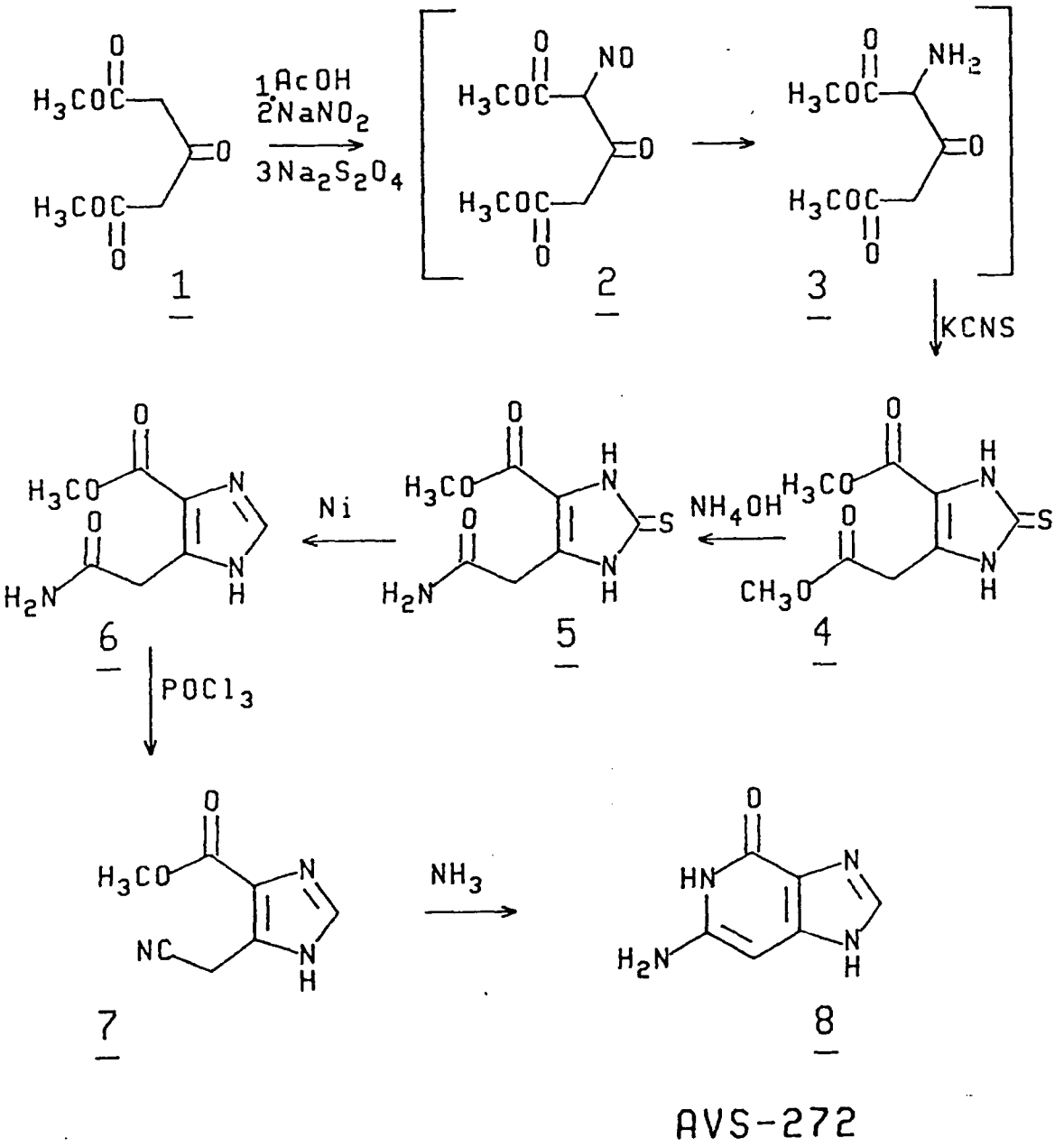
Scheme 7


CHO
OH
OCH3
1
CHO
OSi
OCH3
3
CH2OH
OSi
OCH3
4
H3CO
CHO
H3CO
OCH3
6
CH2Br
OSi
OCH3
5
OH
H3CO
H3CO
OCH3
OSi
OCH3
7
OH
H3CO
H3CO
OCH3
OH
OCH3

Scheme 8



Scheme 9

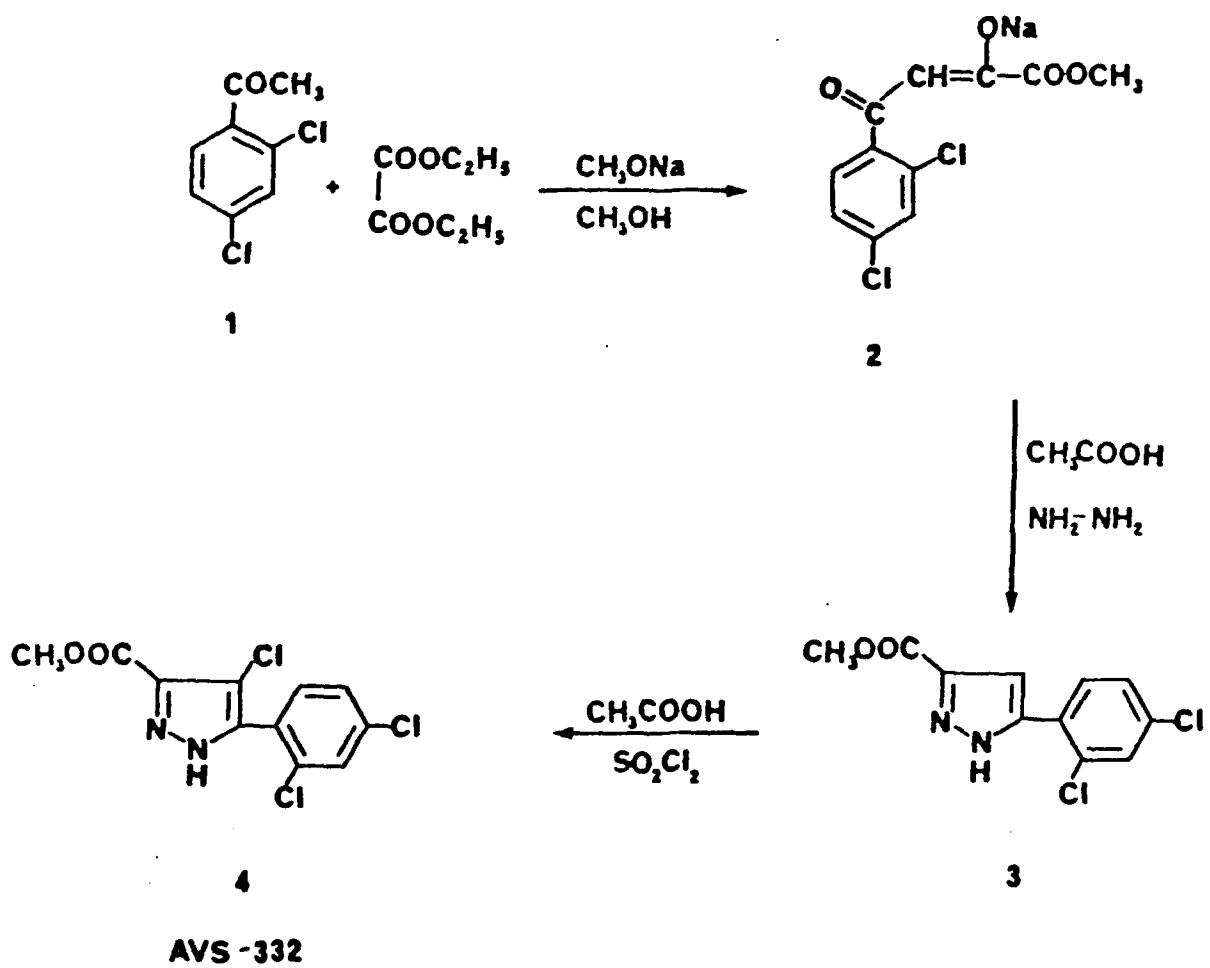


Scheme 10

Synthetic Procedure

Methyl 4-chloro-5-(2,4-dichlorophenyl)-
1(H)pyrazole-3-carboxylate

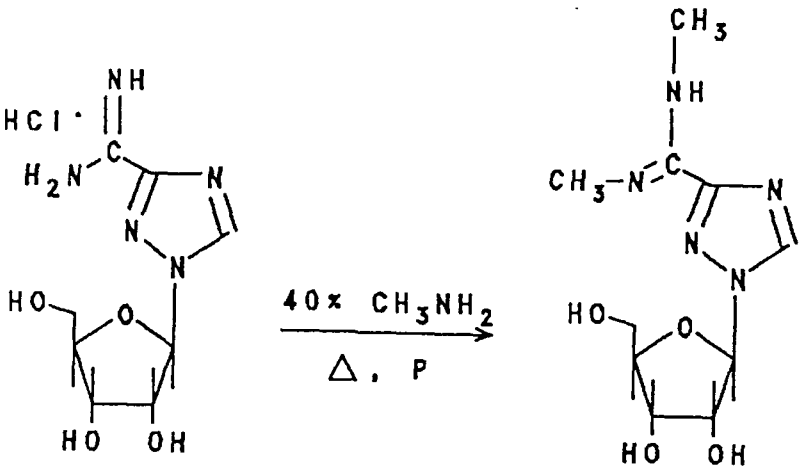
AVS-332



VII. Discussion of two compounds pending submission

A). Presently about 600 g of pure (AVS-1) (Ribavirin) is awaiting shipment to USAMRIID upon receipt of correct analytical results.

B.) Dimethyl ribavirinamidine (AVS 5601), shown below, is still in the synthesis developemental stage. A small sample of the compound will be supplied to USAMRIID to complete all assignment at no cost to the contracting agency.



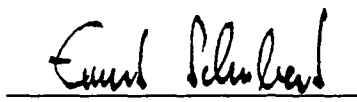
AVS 5601

VIII. Acknowledgements

The personnel assigned to contract DAMD17-85-C-5071 during the contract period were:

1.	Ernst Schubert, Ph.D.	1-17-85	5-31-90
2.	Gary Era, B.S.	4-10-89	5-31-90
3.	Krishna Upadhya, Ph.D.	8-04-86	1-31-90
4.	Valerie Nottingham, B.S.	7-11-88	4-28-89
5.	Bheema Ugarkar, Ph.D.	1-17-85	12-31-86
6.	Jay DaRe, B.S.	1-17-85	6-30-88
7.	Penny Domenico, B.S.	1-17-85	5-31-86

Respectfully Submitted By:
Pharm-Eco Laboratories, Inc.



Ernst M. Schubert, Ph.D
Principal Investigator
(805) 527-3630

IX. Appendix:

Presentations at National and International Meetings.

a. Second International Conference on Antiviral Research.
Williamsburg, Virginia, April 10-14 1988.
"Anti-RNA-Viral Activities of Phenanthridones Related to Narciclasine." B. Gabrielsen, M.A. Ussery, P.G. Canonico, G.R. Pettit, E.M. Schubert, R.W. Sidwell, USAMRIID, Arizona State University, Pharm-Eco Laboratories and Utah State University.

b. Third Chemical Congress Of North America.
Toronto, Canada, June 5-10, 1988.
"Antiviral Structure/Activity Study of the Phenanthridone Alkaloids: Pancretistatin, Narciclasine and Related Compounds." B. Gabrielsen, M.A. Ussery, P.G. Canonico, E.M. Schubert, G.R. Pettit, W.M. Shannon.

c. American Chemical Society 196th National Meeting.
Los Angeles, California, September 25-30, 1988.
"Preparation and Antiviral Evaluation of Deoxygenated Ribavirin and Tiazofurin Derivatives." K.G. Upadhya, J. Da Re, E.M. Schubert, B.J. Gabrielsen.

d. Eastern Analytical Symposium.
New York, N.Y., 1989
"Structural Confirmation by 500 MHz Spectroscopy of an Analogue of the Amaryllidaceae Alkaloids, Narciclasine and Pancratistatin." Bjarne Gabrielsen, Department of Antiviral Studies, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21701, Gwendolyn N. Chmurney, Program Resources, Inc., NCI-FCRF, P.O. Box B, Frederick, MD 21701, Ernst M. Schubert, Pharm-Eco Laboratories, Simi Valley, CA. 93065 and G. Robert Pettit, Cancer Research Institute, Arizona Sate University, Tempe, AZ 85287.

e. Third International Conference on Antiviral Research.
Brussels, Belgium, April 22-27, 1990.
"Antiviral (RNA) Evaluation and Synthesis of a Series of Amaryllidaceae Alkaloids and Related Substances."
B. Gabrielsen, G.R. Pettit, S.B. Singh, T.P. Monath, J.W. Huggins, M.J. Phelan., D. Kefauver, E.M. Schubert, J.H. Huffman, R.W. Sidwell, W.M. Shannon, and J.J. Kirsi, U.S. Army Medical Research Institute, Arizona State University, Tempe, AZ, U.S.A.; Pharm-Eco Laboratories, Simi Valley, CA, U.S.A.; Utah State University, Logan, UT, U.S.A.; Southern Research Institute, Birmingham, AL, U.S.A.

B: Reprints of Publications.

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SYNTHESIS

Journal of
Synthetic Organic
Chemistry

1987
No. 8
August

With Compliments of the Author.

GEORG THIEME VERLAG · STUTTGART · NEW YORK

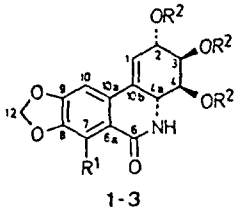
Improved Synthesis of Lycoricidine Triacetate

Bheemarao G. Ugarkar, Jay DaRe, Ernst M. Schubert*

Pharm-Eco Laboratories, Inc., 2355 Chain Drive, Simi Valley, CA 93065, U.S.A.

The synthesis of lycoricidine triacetate by a modified pathway is described. In this preparation, catalytic amounts of osmium tetroxide are used to stereospecifically introduce two hydroxy groups, rendering the title compound via two novel intermediates.

Lycoricidine (1) and lycoricidinol (2), two constituents found in *Amaryllidaceae* plants, show strong growth-inhibiting action in the rice seedling test, and they exhibit anti-tumor activity against Ehrlich carcinoma.[1] Such antimitotic behavior prompted further investigations to establish their configurations and conformations,[1] which were subsequently confirmed by total synthesis.[2,3]



1-3

	R^1	R^2
1	H	H
2	OH	H
3	H	Ac

During recent *in vitro* testing of lycoricidine triacetate (3) it displayed antiviral activity,[4] and a proposed *in vivo* study necessitated the preparation of a larger quantity of 3. The isolation of lycoricidine from plant material is impractical since its abundance in fresh bulbs of *Lycoris* is ~0.00032%, while a reported synthesis produced lycoricidine in a 21-step procedure with 1.5% overall yield, starting with piperonal.[3] After establishing the desired phenanthridone intermediate 4 following a twelve-step procedure, acetylation and removal of the tetrahydropyranyl group is followed by stereospecific *cis*-hydroxylation with equimolar amounts of osmium(VIII) oxide to yield intermediate 5.

The two *cis*-hydroxy groups in 5 are protected as the isopropylidene acetal to allow for the introduction of a double bond between C-1 and C-10b by dehydration. Acetylation, following removal of the isopropylidene group, renders lycoricidine triacetate in 26% yield, based on intermediate 4.

The improved preparation of 3, as shown in the scheme, starts with the direct *cis*-hydroxylation of 4 by utilizing only catalytic amounts of osmium(VIII) oxide, according to a method described in Lit.[5] to yield trihydroxy compound 6. The absence of isomers in the oxidation mixture prior to work-up, as indicated by TLC, attests to the applicability of osmium(VIII) oxide as a highly stereoselective reagent.

After acetylation of the three hydroxy groups in 6, the resulting *O*-tetrahydropyranyl derivative 7 is hydrolyzed without prior purification to render intermediate 8, which upon dehydration yields lycoricidine triacetate in 37% overall yield, starting with 4.

Spectral and analytical data confirm the structural assignments of 3 and, based on 400 MHz ^{1}H-NMR, ^{13}C-NMR, COSY, and HOMCOR spectra, the spatial arrangement at the four chiral carbons is in agreement with the absolute structure assigned to lycoricidine. Two different melting points have been reported for lycoricidine triacetate, depending on its origin,[3] but such a difference could be a result of the mode of recrystallization to obtain a product of differing crystal structure. Hydrolysis of the prepared lycoricidine triacetate, according to Lit.[3], produces lycoricidine (1), whose spectral and analytical data agree with the reported values for lycoricidine of natural origin.

Presently, lycoricidine triacetate (3) is being screened for its *in vivo* antiviral activity, and if proven useful as an antiviral agent, it can readily be prepared according to the reported modifications. They make the procedure economically more attractive for scale-up work because they reduce the number of required steps, thus improving the overall yield, while significantly reducing the hazards of handling and disposing of large amounts of highly toxic osmium oxides.[6]

All solvents used were of reagent grade. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. TLC was run on Silica Gel GF plates (Analtech, Newark, Del.) where the products were visualized by UV-absorbance, or by iodine stains. Microanalyses were performed by MHW Laboratories, Phoenix, Arizona. Mass spectral analyses were taken on a Varian Modell 311 A spectrometer by Dr. K.H. Schram, College of Pharmacy, University of Arizona, Tucson, AZ. IR spectra were obtained on a Beckman AccuLab 2, and UV spectra were recorded on a Beckman Model 25 spectrophotometer. In addition to the standard NMR spectra, taken on a Varian EM 390 spectrometer, 400 MHz ^{1}H-NMR, ^{13}C-NMR, attached proton test (ATP), homonuclear correlation (COSY), and heteronuclear correlation (HETCOR) spectra were recorded by SRI International, Menlo Park, California.

4aH-,1H-*trans*,2H-*cis*,3H-*trans*,4H-*trans*,10bH-*trans*-1-(2-Tetrahydropyranyloxy)-2,3,4-trihydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5*H*)phenanthridone (6):
To a solution of *N*-methylmorpholine *N*-oxide[7] (16.0 g, 0.137 mol) in *t*-BuOH (50 mL), acetone (50 mL), and H_2O (20 mL), osmium tetroxide

(260 mg, 1 mmol) is added. To this reaction medium is added a solution of phenanthridone derivative 4[2] (28.8 g, 80 mmol) in *t*-BuOH (700 mL) over a 10 min period, followed by continued stirring for 48 h. Decolorizing carbon is added to the dark solution, and after stirring for 3 h at room temperature the mixture is filtered through a Celite bed. The resulting pale-yellow solution is evaporated under reduced pressure. The remaining oil is triturated with EtOH (25 mL), and the resulting crystalline material is collected by filtration. The solid is suspended in H_2O (100 mL). This suspension is stirred for 1 h, and filtered. The off-white solid is washed with H_2O, and air-dried to give trihydroxy compound 6; yield: 20.0 g (63%); m.p. 222–223°C; TLC ($CHCl_3$/MeOH 6:1), Rf: 0.60.

$C_{19}H_{23}NO_8$	calc.	C 58.01	H 5.89	N 3.56
(393.4)	found	58.42	5.96	3.62

IR (KBr): ν = 3600–3200 (br), 2970, 1665, 1610, 1495, 1460, 1390, 1355, 1260, 1075, 1035 cm^{-1}.

^{1}H-NMR (DMSO-d_6/TMS): δ = 1.4 (br.s, 9H, tetrahydropyran); 3.1–4.5 (m, 8H, $5H_{aliph}$ + 3OH); 4.9 (s, 1H, H-4a); 6.15 (D, 2H, J = 2.5 Hz, $-CH_2-$); 6.8 (s, 1H, NH); 7.05 (s, 1H, H-10); 7.4 (s, 1H, H-7).

4aH-*v*,1H-*trans*,2H-*cis*,3H-*trans*,4H-*trans*,10bH-*trans*-1-Hydroxy-2,3,4-triacetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5*H*)phenanthridone (8):

A mixture of trihydroxy compound 6 (18.6 g, 0.047 mol), pyridine (200 mL), and acetic anhydride (200 mL) is stirred at room temperature overnight. Acetic anhydride and pyridine are evaporated under reduced pressure, followed by evaporation with EtOH to remove traces of pyridine. The residual material is soaked in EtOH (50 mL) and chilled. The resulting crystalline material is collected by filtration, washed with EtOH (50 mL), and air-dried; yield of intermediate 7: 22.0 g; m.p. 275°C.

Without further characterization, intermediate 7 is suspended in EtOH (500 mL), *p*-toluenesulfonic acid (500 g) is added, and the mixture is kept at reflux for 2 h. Upon cooling, the crystalline material is filtered, washed with cold EtOH (2 × 25 mL), and dried in air; yield of 8: 15.0 g (73%); m.p. 303–304°; TLC ($CHCl_3$/MeOH 6:1), Rf: 0.75.

$C_{20}H_{21}NO_{16}$	calc.	C 55.17	H 4.86	N 3.21
435.4	found	54.95	4.90	3.13

IR (KBr): ν = 3460, 3180, 3070, 2965, 1740, 1670, 1480, 1450, 1360, 1250, 1215, 1040, 920 cm^{-1}.

^{1}H-NMR (DMSO-d_6/TMS): δ = 2.05 (s, 6H, 2 acetyl); 2.15 (s, 3H, 1 acetyl); 3.1–5.5 (m, $7H_{aliph}$); 6.15 (s, 2H, $-CH_2-$); 6.9 (s, 1H, NH); 7.35 (s, 1H, H-10) 7.9 (s, 1H, H-7).

Lycoricidine Triacetate [4aH-*v*,1H-*trans*,2H-*cis*,3H-*trans*,4H-*trans*,10bH-*trans*,2,3,4-Triacetoxy-8,9-methylenedioxy-2,3,4,4a-tetrahydro-6(5*H*)-phenanthridone] (3):

Intermediate 8 (7.0 g, 18 mmol) is dissolved in pyridine (70 mL) while stirring; upon cooling of the mixture, thionyl chloride (10 mL) is added over a 20 min period. Stirring is continued overnight while the mixture is allowed to warm up to room temperature. CH_2Cl_2 (500 mL) is added, and the solution is washed with H_2O (2 × 500 mL), with 10% hydrochloric acid (2 × 250 mL), and H_2O (2 × 500 mL). The organic phase is dried (Na_2SO_4) and evaporated under reduced pressure to yield a white solid. Upon trituration with MeOH (40 mL), the crystalline material is filtered, washed with MeOH (2 × 20 mL) and dried in air. This product is recrystallized from CH_2Cl_2/MeOH, 1:1 (50 mL) to give the pure product 3. From the mother liquor, a second crop is obtained; total yield: 6.0 g (80%); m.p. 266–268° (dec.).

$C_{20}H_{19}NO_9$	calc.	C 57.55	H 4.59	N 3.35
417.3	found	57.46	4.50	3.36

The MS-, IR-, and UV-spectral data all agree with the reported values.[3]

^{1}H-NMR ($CDCl_3$/TMS): δ = 2.09 (s, 3H, acetyl); 2.11 (s, 3H, acetyl); 2.15 (s, 3H, acetyl); 4.65 (d, 1H, J = 10 Hz, H-4a); 5.2–5.4 (m, 3H, H-2, H-3, H-4); 6.10 (s, 3H, H-1, H-12); 7.00 (s, 1H, H-10); 7.35 (s, 1H, NH); 7.50 (s, 1H, H-7).

^{13}C-NMR ($CDCl_3$/TMS): δ = 21.0 (3C, acetyl), 50.1 (C-4a), 68.2 (C-4), 68.6 (C-3), 71.2 (C-2), 102.0 (C-12), 103.4 (C-10), 107.5 (C-7), 117.0 (C-1), 122.5 (C-10b), 130.3 (C-10a), 134.1 (C-6a), 149.2 (C-9), 151.7 (C-8), 164.3 (C-6), 169.5 (C=O), 169.7 (C=O), 170.4 (C=O).

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Preparation of Dimethyl (1-Formylalkyl)phosphonates *via* Singlet Oxygen Adducts

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The nitro group of dimethyl (1-nitromethylalkyl)phosphonates is conveniently converted into a formyl group by reaction with singlet oxygen to give dimethyl (1-formylalkyl)phosphonates. (1-Formylbutyl)diphenylphosphine oxide is prepared by the same reaction.

The conversion of nitroalkanes into carbonyl compounds is a synthetically useful functional group interconversion. The reaction is usually carried out under strongly acidic,[1] basic oxidative,[2–8] neutral oxidative,[9] or neutral reductive conditions.[7,10–12] Surprisingly, our numerous attempts to effect the analogous conversion of organophosphorus compounds having a *P*-(2-nitroalkyl) group under various conditions were unsuccessful. However, we then found that oxidation with ozone conveniently converts [1-(nitromethyl)alkyl]diphenylphosphine oxides into (1-formylalkyl)diphenylphosphine oxides;[13] however, overoxidation is problematic and does not allow for efficient scale-up.

Phosphoric esters are widely known to excert a great variety of vital functions. Some phosphonates having a C-P bond, formally being prepared by replacing the O-atom of a P-O-C linkage of phosphoric esters by the isoelectronic CH_2 group, are also naturally occuring, and their metabolism has been investigated.[14] Phosphonic acid derivatives such as (−)-(1*R*, 2*S*)-1,2-epoxypropylphosphonic acid (phosphonomycin) and dimethyl 1-hydroxy-2,2,2-trichloroethylphosphonate (Dipterex) are active substances as antibiotics and pesticides[15,16] and are therefore of practical importance.

PREPARATION AND ANTIVIRAL ACTIVITY OF SEVERAL DEOXYGENATED RIBAVIRIN AND TIAZOFURIN DERIVATIVES.

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Abstract: Ribavirin and tiazofurin, two nucleosides of known antiviral activity, have been transformed by previously reported methods to yield several deoxy,epoxy, or dideoxy analogues. The deoxygenated derivatives were evaluated for antiviral activity against a host of DNA and RNA viruses; however, no significant *in vitro* activity was detected.

In the past, a number of 2'3'-dideoxynucleosides have been prepared and evaluated for their antiviral activity. Such studies were mostly directed towards suppressing the replication of the human immunodeficiency virus in the treatment of the acquired immune deficiency syndrome (AIDS). 3'-Azido-3'deoxy-thymidine (AZT)[1] and 2'3'-dideoxycytidine (ddCyd)[2] were found to be the most active pyrimidine nucleosides, while recent studies indicate that 2'3'-dideoxyinosine (DDI), a purine riboside derivative, might find wide clinical application in the treatment of AIDS.[3]

Since none of the parent nucleosides such as thymidine, cytidine, or inosine show any noticeable antiviral activity, it was thought that the transformation of a ribonucleosides of known antiviral activity such as ribavirin or tiazofurin[5] into their deoxygenated derivatives would offer the possibility of augmenting their respective biological activities, or enhancing their therapeutic specificity. Analogously, a recent publication by Krawczyk and Townsend[6] reports the synthesis of the 2'3'-dideoxy derivatives of the antibiotics tubercidin, toyocamycin and sangivamycin as examples of biologically active purine nucleosides which were transformed into agents that might demonstrate anti-HIV activity.

Since the preparation of 2',3'-dideoxyribosides as well as those of other sugar-modified nucleosides has been the topic of a number of studies in recent

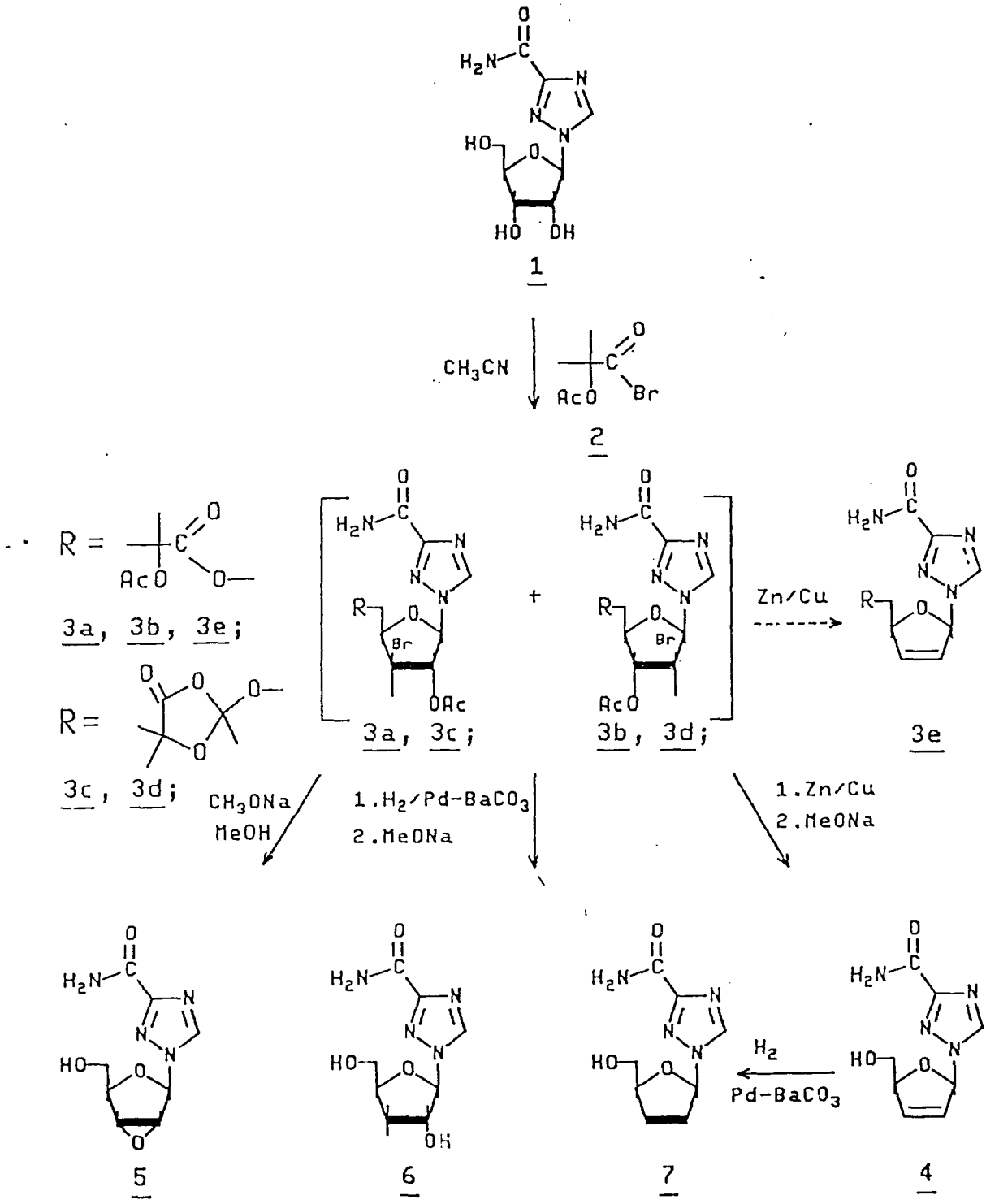
years, there are several methodologies, such as the modified Corey-Winter reaction[7] and other elimination[8] or synthesis methods[9] available to accomplish such transformations. During the course of this study we found that a modified procedure, based on work reported by Robins et al.[10] was best suited for transforming both the N-nucleoside ribavirin and the C-nucleoside tiazofurin into various sugar-modified analogues via a common intermediate (3a-d and 9a-d) by essentially using identical reagents and reaction conditions.

Both ribavirin (1) and tiazofurin (9) were acylated with α-acetoxy-isobutyryl bromide (2), as shown in Schemes 1 and 2, to form a mixture of four possible intermediates, shown by structures 3a-d and 9a-d. This mixture of intermediate isomers was subjected to transformations without further characterization; however, upon careful dehydrohalogenation and purification of either 3a-d or 9a-d without deblocking the 5'-position, the ^{1}H NMR spectrum of the purified product 3e or 9e showed the two α-methyl groups of the side chains as one singlet (6H), indicating the existence of the open chain, and not the sterically rigid dioxolone ring configuration as a possible structure.

The treatment of 3 and 9 with zinc/copper couple and sodium methoxide readily yielded *enes* 4 and 10 respectively, which in turn were readily hydrogenated to form 2'3'-dideoxyribavirin (7) and 2'3'-dideoxytiazofurin (11) in good yield.

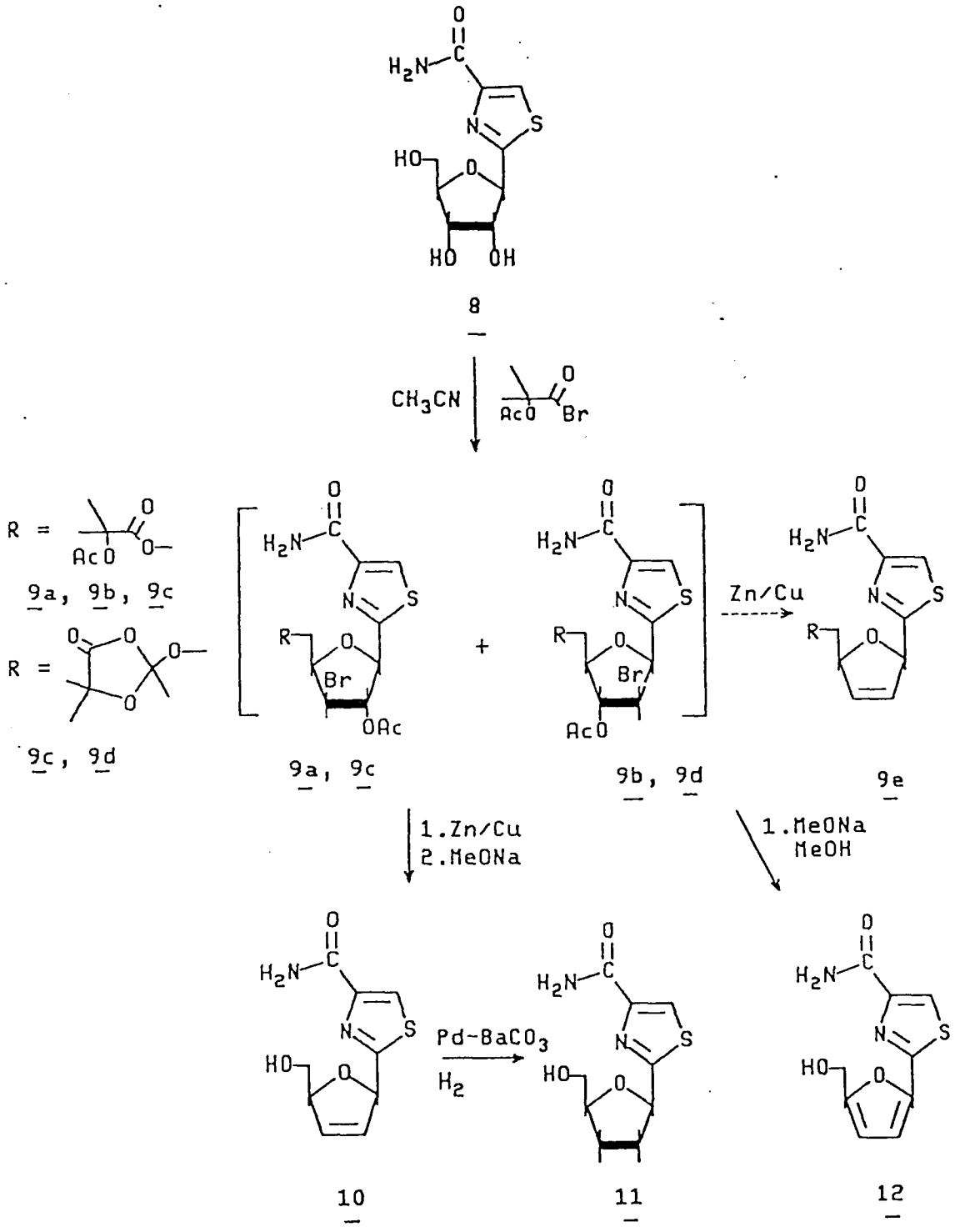
Hydrogenation of 3, followed by deblocking, gave 2'3'-dideoyxribavirin (7). The major product isolated from this reaction, however, was 3'-deoxyribavirin (6), as identified by comparison with data published by Witkowski et al. The treatment of 3 with sodium methoxide in methanol produced 2',3'-anhydroribavirin 5; yet, when the same reaction conditions were applied to 9, it resulted in double elimination and formation of the furan derivative of the thiazole amide 12, first reported by Srivastava et al.[5]

2'3'-Dideoxyribavirin, previously prepared by a different route and shown to be inactive against the HIV virus[12] was still considered a viable candidate to be screened as part of the whole series of obtained compounds against a number of different RNA and DNA viruses, as discussed below.



Ribavirin Series

SCHEME 1



Tiazofurin Series

SCHEME 2

Ribavirin (1) possesses considerable activity in vitro against RNA viruses of the Bunyaviridae family[13,14] (Rift Valley fever, RVF'sandfly fever, SFS, and Punta Toro, PT viruses).[15] Activity has also been demonstrated against the retrovirus human immunodeficiency virus type 1 (HIV-1)[16], the DNA-containing adenovirus type 2 (AD2)[13], and vaccinia virus (VV),[13] and the RNA-containing alphavirus, Venezuelan equine encephalomyelitis virus (VEE)[13,14]. Activity is also present, but to a lesser degree, against RNA viruses of the Flaviviridae family, yellow fever (YF), and Japanese encephalitis (JE) viruses[13,14]. Virtually no activity is observed against vesicular stomatitis virus, VSV (Rhabdoviridae family)[13]. Tiazofurin 8, possesses some activity in vitro against the flaviviruses YF and JE[13,14], lesser activity against the bunyaviruses RVF, PT[13,14] and SFS, and the DNA-containing adenovirus and vaccinia virus[13]. No activity has been reported against HIV, VEE, and VSV[13].

In vitro antiviral activities were determined for the deoxygenated ribavirin analogues 4-7 and tiazofurin analogues 10-12 against human immunodeficiency virus (HIV-1), the RNA-containing bunyaviruses (Rift Valley fever, sandfly fever, and Punta Toro viruses), flaviviruses (Japanese encephalitis and yellow fever viruses), alphavirus (Venezuelan equine encephalomyelitis virus), rhabdovirus (vesicular stomatitis virus), and the DNA-containing adenovirus type 2 and vaccinia virus. The observed antiviral activities are summarized in the accompanying table. Replacement of the ribofuranosyl group in the deoxygenated tiazofurin analogues 10-12 resulted in the loss of all in vitro antiviral activity previously observed for tiazofurin 8 against the flaviviruses, bunyaviruses and DNA viruses, and vaccinia and adenovirus type 2. Compounds 10-12 were also inactive against HIV-1, VEE, and VSV.

Replacement of the ribofuranosyl group of ribavirin 1 by 2',3'-dideoxy (7), 3'-deoxy (6), or 2',3'-anhydro (5) ribofuranosyl moieties resulted in elimination of all antiviral efficacy against HIV-1, vaccinia and adenoviruses, flaviviruses (JE, YF), Venezuelan equine encephalomyelitis (VEE), bunyaviruses (PT, SFS) and no resulting activity against vesicular stomatitis virus (VSV).

2',3'-Dideoxy-2',3'-didehydro ribavirin 4 retained some efficacy only against the bunyaviruses (RVF, PT, SFS) and vaccinia virus, however the level of efficacy in vitro was greatly reduced compared to that of ribavirin. Similar reduced activity was also observed against Rift Valley fever virus by 5-7. Plaque reductions of 80% (@ 100 ug/mL), 59%, 76% and 94% were observed for 4-7 respectively against RVF virus in Vero cells at 250 ug/mL. However the activities of 4-7 against RVF could not be separated from the accompanying Vero cell toxicity. 2',3'-Dideoxyribavirin 7 and 3'-deoxyribavirin 6 were evaluated further in the murine model of Rift Valley fever virus[17]. Doses of 25, 125 and 250 mg/kg/day were administered subcutaneously in 10% DMSO-PBS or saline on days -1 to +3. No beneficial effects were observed in terms of increased survival numbers or times, nor were the compounds toxic at these doses (virus ratings, VR, 0.96-0.99). As a positive control, ribivirin at doses of 100 and 200 mg/kg/day protected 100 % of the RVF-infected mice (VR = 5.4 and 6.1 respectively).

IN VITRO ANTIVIRAL TESTING DATA[e]

Compound	Virus	ID_{50}[a]	MTC[b]	TI[c]	[d]TI[+]
4	RVF	61	<100	1.6	6.6
4	SFS	5	10	2.0	5.6
4	PT	28	32	1.1	6.3
4	YF	73	10	0.1	1.2
4	VV	36	100	2.8	7.5
5	RVF	149	250	1.7	6.6
6	RVF	117	250	2.1	6.6
7	RVF	101	<250	2.5	6.6

[a] 50% Viral inhibitory dose, µg/ml
[b] Minimum Toxic Concentration, µg/ml
[c] Therapeutic Index, TI = MTC_{50}/ID_{50}
[d] Positive drug controls : ribavirin (RVF, SFS, PT), selenazofurin (YF, JE), adenosine arabinoside, ara-A (VV)
[e] Vero cells

EXPERIMENTAL SECTION

Analytically pure ribavirin and tiazofurin were provided by US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, MD.

Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. The utilized 2n/Cn couple contained 5% copper. Silica gel used for chromatography was flash grade (Aldrich, 260-400 mesh), and thin-layer chromatography (TLC) was performed on prescored silica gel plates GHLF, 250 microns (Analtech Corp., Newark, DE.) with 6:1 dichloromethane-methanol as developing solvent. TLC plates were sprayed with 10% methanolic sulfuric acid after elution and heated to visualize the compounds. IR spectra were recorded using a Beckman AccuLab 2 spectrophotometer, and elemental analyses were performed by MHW Laboratories, Phoenix, Arizona.

All of the NMR spectra with the exception of 12 were obtained on a Varian VXR500S NMR spectrometer equipped with a SUN 4/110 acquisition computer and data station. The following 90° pulse widths were used for 1D and 2D data acquisition: proton, observe = 14.0 μsec, Waltz decouple = 89.3 μsec; carbon, observe = 15.0 μsec, Waltz decouple = 30.8 μsec, 2D 90° PW = 29 μsec. For 1D experiments, the Ernst angle was used for acquisition. Heteronuclear multiple quantum coherence (hmqc) standard pulse sequence from Varian software was used to obtain directly bonded, indirectly detected proton-carbon connectivities (ref. Bax, $^{1}J_{CH}$ = 150 Hz)[18]. Heteronuclear multiple bond connectivity (hmbc) was modified from Varian software according to Bax[18] and used to detect long range proton-carbon connectivities (Jnxh - 8 Hz). Standard Varian COSY was used for proton-proton connectivity determination. Zero field decoupling (ZFD) and modified Varian software were used to obtain chemical shift and coupling information.

Spectra of 12 were obtained on a Varian XL200 with an ADVANCE data system operating at 200.1 MHz. The following 90° pulse widths were operational: proton,observe = 23.5 μsec, Waltz decoupling = 79.2 μsec; carbon, observe = 9 μsec. The Ernst angle was used for 1D data acquisition.

Definition of J coupling notations: capital letters in ^{13}C coupling

patterns refer to directly bonded $^1J_{C-H}$ while lower case letters refer to J coupling over more than one bond. For example, Ddt (156, 2.0, 10.8) means that the carbon in question has a directly bonded J_{CH} of 156 Hz, a long-range coupling to one proton of J = 2.0 Hz and a long range coupling to two protons of J = 10.8 Hz, s = singlet, d = doublet, t = triplet, q = quartet, b = broad, cm = complex multiplet.

The chemical shifts in the proton spectra are referenced from tetramethylsilane (TMS) set equal to 0 ppm. The chemical shifts in the carbon spectra are referenced with respect to dimethylsulfoxide-d_6 ($DMSOd_6$) set equal to 39.5 ppm from TMS.

In vitro antiviral activity was determined in terms of therapeutic index by observing inhibition of viral cytopathic effect (CPE)[13,15,19-22] except for RVF virus which was determined by plaque reduction assays [14]. The 50% inhibitory dose is that drug dose causing a 50% inhibition of CPE or plaque number. The minimum cytotoxic concentration (MTC) is that drug concentration at which 50% of the cells showed cytotoxic effects. The in vitro therapeutic index (TI, proportional to in vitro activity) was calculated by dividing the MTC by the ID50. Compounds were evaluated for therapeutic efficacy in Rift Valley fever-infected mice according to the procedure of Peters et al[17]. The in vivo virus rating, VR, was calculated by dividing the geometric mean time to death of drug-treated, infected animals by that for untreated, infected animals.

1-(2,3-Dideoxy-ß-*D*-glycero-pent-2-enofuranosyl)-1,2,4-triazole-3-carboxamide (4). (2'3'-Dideoxy-2',3'-didehydroribavirin):

Ribavirin (1) (19.5 g, 80 mmol) was dissolved in acetonitrile (200 mL) containing water (1.44 mL, 80 mmol). To this solution was added α-acetoxyisobutyryl bromide (2) (49.4 g, 36 mL, 240 mmol) in one portion, and stirring was continued at room temperature for two hours. After adding 5% sodium bicarbonate solution (200 mL) the mixture was extracted with ethyl acetate (2 x 200 mL), and the organic phase was washed with sodium bicarbonate solution and with brine. After evaporation of the solvent under reduced pressure a highly

viscous foam was obtained, which was dissolved in tetrahydrofuran (600 mL). Zinc/copper couple (80 g) was added, followed by ammonium chloride (50 g) and the reaction mixture was stirred for two hours when the temperature reaches 40°. The zinc/copper couple was filtered off, washed with ethyl acetate and the organic layer was washed with a 5% aqueous solution of ethylenediamine tetraacetic acid tri-sodium salt, followed by washings with bicarbonate (100 mL) and brine (200 mL).

The solvent was removed under reduced pressure, the residue was dissolved in methanol (200 mL) and sodium methoxide (0.5 g) was added to adjust the pH to 9.5. After stirring for three hours a solid started to precipitate. The solvent volume was reduced to half its volume, the precipitate was collected by filtration and recrystallized from methanol-ethyl acetate.

Yield 7.0 g (42%); m.p. 152-153°; IR (KBr): 3400-3050; 1750; 1480; 1750; 1480; 1270; 1190; 1070; 840; 780 cm^{-1}. ^{1}H-NMR: (DMSO-d_6) δ 8.75(s, 1, C_5H); 7.82 and 7.63 (each singlets, 1H each, NH); 6.85(td,1,H-1',J(1',2') = 1.6 Hz, J(1',4') = 2.4 Hz); 6.51 (td, 1,[a]H-3',J(3',4') = 1.7 Hz, J(3',2')=6.1 Hz); 6.13 (ddd, 1,[a1]H-2',J(2',1') = 1.5 Hz, J(2',4') = 2.3 Hz, J(2',3') = 6.0 Hz); 4.914 (ddddd, 1, H-4', J(4',1') = 2.4 Hz, J(4',3') = 1.7 Hz, J(4',2') = 2.3 Hz, J(4,5'a,b) = 4.2, 4.8 Hz, 4.908 (4, 1, 5'-OH, J(OH-5' = 5.6 Hz; 3.48, 3.55 (AB of ABXY, 2, H-5'a,b,J(gem) = 11.6, 11.7, J(5',a,b,4')=4.2, 4.8Hz, J(4', OH) = 5.6 Hz);

^{13}C-NMR: (DMSO-d[6]): δ 160.32 (Sq, C=O, J = 1.2 Hz); 156.75 (Sdd, C-3, J = 8.5, 11.7 Hz); 144.13 (Dd, C-5, J = 214.7, 2.7 Hz); 134.58 (Dtdd, C-3', J = 172.3, 4.0, 2.4, 7.4 Hz); 124.56 (Dtd, C-2', J = 176.9, 4.1, 2.5 Hz); 93.24 (Dtd, C-1', J = 171.1, 10.6, 3.6 Hz); 88.61 (Dddt, C-4', J = 149.8, 8.8, 11.3, 2.2 Hz); 62.84 (T, C-5', J = 141.0 Hz);

TLC: R_f 0.7. Anal. Calcd. for $C_8H_{10}N_4O_3$: C, 45.61; H, 4.79; N, 26.66;. Found: C, 45.85; H, 4.76; N, 26.85.

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[1]Assigned from coupled ^{13}C spectrum through HMQC.

1-(2,3-Dideoxy-ß-D-glycero-pentofuranosyl)-1,2,4-triazole-3-carboxamide (7) (2',3'-Dideoxyribavirin):

Ribavirin-2'-ene (4) (2.3 g, 11 mmol) was dissolved in methanol (100 mL), and palladium on barium carbonate (500 mg) was added. The mixture was hydrogenated at room temperature and atmospheric pressure for 3 hours. The catalyst was filtered off on a sintered glass funnel, the filtrate was evaporated to dryness under reduced pressure, and the residue was recrystallized from methanol (50 mL) to yield 1.8 g (77%) of dideoxyribavirin, m.p. 153-154°.(lit[12] 154°C) IR (nujol): 3000-2800 (br); 1690; 1460; 1370 cm^{-1}.

^{1}H NMR:[12] (DMSO d_6) 12: δ 8.81 (s, 1, $C_5\underline{H}$); 7.79, 7.59 (each singlet, 1, NH); 6.16 (dd, 1, H-1', J(1'-2'a,b) = 2.6, 6.5 Hz); 4.88 (t, 1, 5'-OH, J = 5.6 Hz); 4.15 (dddd, 1, H-4', J = 5.2, 4.5, 6.0, 9.2 Hz); 3.56 (ddd, 1, H-5'a, J = 11.7, 4.2, 5.7 Hz); 3.47 (dt, 1, H-5'b, J = 11.7, 5.3 Hz); 2.38 (cm, 2, H-2'a,b); 1.98 (cm, 2, H-3'a,b).

^{13}C-NMR: (DMSO d_6): δ 160.37 (S, C=O); 156.84 (Sdd, C-3, J = 8.2, 11.4 Hz); 143.88 (Dd, C-5, J = 214.2, 1.8 Hz); 88.61 (Dcm, C-1', J_{ch} = 170.1 Hz); 82.84 (Dcm, C-4', J_{ch} = 146.3 Hz); 62.86 (Td, C-5', J = 139.8, 4.7 Hz); 31.90 (Tt, C-3', J = 134.1, 3.1 Hz); 25.32 (Tcm, C-2', J_{ch} = 133.0 Hz).

TLC: Rf 0.65. Anal. Calcd. for $C_8H_{12}N_4O_8$: C, 45.27; H, 5.70; N, 26.40. Found: C, 45.26; H, 5.72; N, 26.36.

3'-Deoxyribavirin (6):

Ribavirin (1) (4.88 g, 20 mmol) was dissolved in acetonitrile (60 mL) and α-acetoxyisobutyryl bromide (2) (9 mL, 50 mmol) was introduced in one portion. The reaction mixture was stirred for two hours at room temperature, then ethyl acetate (300 mL) was added to the clear solution. The organic layer was washed with 5% sodium bicarbonate solution (2 x 50 mL), the bicarbonate phase was washed with ethyl acetate (100 mL), and the combined organic phase was washed with water (2 x 50 mL) and saturated brine (50 mL). The ethyl acetate solution was dried over sodium sulfate, and the solvent was evaporated under reduced pressure to yield 9.2 g of (3) as a viscous oil.

The crude material was dissolved in dry methanol (200 mL), then triethylamine (3 mL) was added, followed by 5% palladium on barium carbonate (2 g). The reaction mixture was hydrogenated at room temperature and atmospheric pressure for two hours, then stirring was continued for four more hours. The catalyst was filtered off, the solvent was removed under reduced pressure and the residue was vacuum-dried. After dissolving the residue in methanol (200 mL) sodium methoxide (1.5 g) was added, and after two hours TLC indicated the completion of deblocking, showing the presence of two products: the spot at R_f 0.7 indicated dideoxy-didehydro-ribavirin (4) while the major product at R_f 0.3 represented 3'-deoxy-ribavirin (6).

The solvent was evaporated under reduced pressure, the residue was loaded onto a silica gel column and eluted with methylene chloride, gradually increasing its polarity by adding methanol. Collecting the fractions containing the two compounds, 0.5 g of dideoxy-didehydro-ribavirin (4) and 2.1 g (47%) of 3'-deoxy-ribavirin (6) was obtained, m.p. 141-142°.(lit[11] 141-142°).

IR (nujol): 3400-3000 (br); 2950; 1680; 1600; 1455; 1300; 1110; 1080; 710 cm^{-1}.

^1H NMR (DMSO-d_6): δ 8.87 (d, 1, $C_5\underline{H}$, $^4J(5,1') = 0.2$ Hz); 7.85 (bs, 1, NH); 7.64 (bs, 1, NH); 5.86 (d, 1, H-1', $J(1'\text{-}C_5H) = 0.7$ Hz); 5.74 (bd, 1, 2'-OH, J = 3.8 Hz); 4.98 (bs, 1, 5'-OH); 4.47 (cm, 1, H-4'); 4.43 (bdt, 1, H-2', J(2'-OH) = 5 Hz, J(2',3') = 9.8 Hz); 3.65 and 3.52 (both dd, 1 each, H-$5_{a,b}$', $J(5'^a,5'_b) = 11.7$ Hz, J(5',4') = 2.4, 4.5 Hz); 2.12 and 1.90 (both ddd, 1 each, H-$3'_{a,b}$, J(gem) = 13.2 Hz, J(3', 2') = 10.1 Hz, J(3',4') = 1.5, 5.1 Hz.

^{13}C-NMR (DMSO-d_6): δ 160.59 (cm, C=O); 157.26 (Sdd, C-3, ^3J = 8.5, 11.6 Hz); 144.27 (Dd, C-5, J = 215.6, 2.0 Hz); 94.67 (Dcm, C-1', $^1J_{CH}$ = 170.3 Hz); 82.37 (cm, C-41, $1J_{CH}$ = 148.9 H_3); 75.50 (Dcm, C-2', J_{CH} = 153.2 Hz); 62.59 (Td, C-5', J = 140.2, 3.7 Hz); 33.77 (Tcm, C-3', J_{CH} = 132.3 Hz).

TLC: Rf 0.3. Anal. Calcd. for $C_8H_{12}N_4O_4$: C, 42.10; H, 5.30; N, 24.55. Found: C, 42.22; H, 5.41; N, 24.35.

1-(2',3'-Anhydro-ß-*D*-ribofuranosyl)-1,2,4-triazole-3-carboxamide (5)

Ribavirin (1) (2.44 g, 10 mmol) was dissolved in acetonitrile (30 mL) containing water (0.18 mL). While stirring α-acetoxyisobutyryl bromide (2) (4.5 mL, 30 mmol) was added in one portion. After 2 h at room temperature ethyl acetate (200 mL) was added, the solution was washed with sodium bicarbonate solution 5% (2 x 50 mL), the bicarbonate solution was extracted with ethyl acetate (100 mL) and the combined ethyl acetate extracts were washed with water (2 x 50 mL) and saturated brine solution.

The organic phase was dried over sodium sulfate, filtered, and, upon evaporation of the solvent, 5 g of crude material was obtained. The crude product (5 g) was dissolved in 1 M methanolic sodium methoxide solution (40 mL) and stirred for two hours, during which time a solid separated from solution. The solid was collected by filtration and recrystallized from water to yield 1.8 g (80%) of final product, m.p. 233-235°. IR (nujol): 3430; 3265; 3000-2800 (br); 1690; 1600; 1460; 1375; 1290; 1190; 1070; 1030; 970; 860; 830 cm^{-1}.
^{1}H NMR (DMSO-d_6): δ 8.847 (s, 1, C_5H , ^4J < 0.4 Hz if present); 7.879 (s, 1, NH); 7.705 (s, 1, NH); 6.281 (s, 1, H-1', J < 0.7 Hz if present); 4.985 (t, 1, 5'-OH, J = 5.5 Hz); 4.30 (dd, 1, H-2', ^3J(2',1') = 0.5 Hz, J(2',3') = 2.7 Hz); 4.21 (d, 1, H-3', J (3',2') = 2.7 Hz, coupling was small to H-4' if present); 4.18 (dd, 1, H-4', J(4',5'$_{a,b,}$) = 5.8, 6.8 Hz); 3.63 (ddd, 1, H-5'$_a$) and 3.47 (ddd, 1, H-5'$_b$), J(5'$_a$,5'$_b$) = 11.4, 11.3 Hz, J(5'$_{a,b,}$ OH) = 5.6, 5.7 Hz, J(5'$_{a,b,}$4') = 6.8, 5.6 Hz.
^{13}C-NMR (DMSO-d^6): δ 160.11 (Sdd, C=O, ^2J(C-N-H) = 1.1, 2.2 Hz; 157.45 (Sdd, C-3, ^3J = 8.8, 11.5 Hz); 145.56 (Dd, C-5, J = 215.7, 1.6 Hz); 85.26 (Ddd, C-1', J = 171.6, 5.9, 10.6 Hz); 81.21 (Ddcm, C-4', J = 151.9, 11.7, complex multiplet); 60.52 (Tcm, C-5', J = 142.1, complex multiplet); 57.43 (D of pentuplets (to H-5'$_{a,b,}$ H-4' and H-2') C-3', J -193.4, 4.6 Hz); 57.16 (Dt, C-2', J = 197.8, 4.0 Hz).
TLC: Rf 0.32. Anal. Calcd. for $C_8H_{10}N_4O_4$: C, 42.47; H, 4.45; N, 24.77. Found: C, 42.46; H, 4.51; N, 24.88.

2-(2,3-Dideoxy-ß-*D*-glycero-pent-2-enofuranosyl)-thiazole-4-carboxamide (10) (2',3'-dideoxy-2',3'-didehydrotiazofurin):

Tiazofurin (8) (5.12 g, 20 mmol) was suspended in acetonitrile (60 mL) containing water (0.36 mL), and α-acetoxyisobutyryl (2) bromide (9 mL, 60 mmol) was added in one portion. After stirring at room temperature for three hours ethyl acetate (200 mL) was added, and the solution was washed with 5% sodium bicarbonate (2 x 50 mL). The aqueous layer was extracted with ethyl acetate (100 mL), the combined organic layers were washed with water (2 x 50 mL) and brine (50 mL), followed by drying over sodium sulfate.

The solvent was evaporated under reduced pressure and the thus obtained foam was dissolved in tetrahydrofuran (200 mL). Zinc-copper couple (25 g) and ammonium chloride (12 g) were added and the mixture, initially at 40°C, were stirred while allowing the temperature to adjust to room temperature. After 2.5 hours, the Zn/Cu-couple was filtered off, the solvent was evaporated under reduced pressure, and the residue was taken up in ethyl acetate (300 mL). The solution was washed with a 5% EDTA tri-sodium salt solution (2 x 50 mL), the aqueous phase was extracted with ethyl acetate (100 mL) and the combined organic layers were washed with water (100 mL) and brine (50 mL). After drying over sodium sulfate the solvent was evaporated under reduced pressure, the residue was dissolved in methanol (100 mL), and sodium methoxide (0.5 g) was added to a pH of 10. After stirring for two hours TLC indicated complete disappearance of starting material and Amberlite H^+ resin was added to neutralize the medium. The resin was filtered off, and the solvent was evaporated under diminished pressure. The residue was chromatographed on a silica gel column with dichloromethane/5% methanol as eluant. Removal of solvent in vacuo from fractions containing 10, followed by recrystallization from ethyl acetate gave 3.9 g (86%) of 10, m.p. 120-121°. IR (nujol): 3460; 3300; 3000(br); 2950; 2840; 1645; 1570; 1450; 1360; 1280; 1070; 1030 cm^{-1}.

^{1}H NMR: (DMSO d_6) δ 8.2 (d, 1, C_5H, ^{5}(5-1') = 0.4 Hz); 7.7 and 7.6 (each bs, 1 each, NH, slight exchange with D_2O); 6.17 and 6.13 [(AB of ABXY, 2, H-3' and H-2' respectively;[a] J(2',3') = 6.1 Hz, J(2', 1') = 1.8 Hz, J(2',4') = 2.1 Hz,

J(3',4') = 1.4 Hz, J(3',1') = 2.3 Hz]; 6.02 (dddd, 1, H-1', J = 0.4, 1.6, 2.1, 3.8 Hz; 4.93 (t, 1, 5'-OH, exchanged with D_2O, J = 5.6 Hz); 4.92 (dddt, 1, H-4', J = 1.5, 2.3, 3.8, 5.4 Hz, couplings to H-3', H-2', H-1' and H-5'a,b respectively); 3.59 and 3.53, (AB of ABXY, 2, H-5'a,b; J(gem) = 11.2 Hz, J(5'-4') = 5.4, 5.4 Hz, J(5'-OH) = 5.7, 5.4 Hz.

^{13}C-NMR: (DMSO d_6) δ 173.1 (Stdd, C-2, J = 1.7, 5.2, 7.2 Hz)[a2]; 162.3 (Sd, C-4, $^2J_{CH}$ = 1.7 Hz); 150.2 (Sdd, C=O, J = 4.5, 6.8 Hz); 130.3 (Dsextets, ^{b3}C-2', J = 171.6, 3.6 Hz); 128.6 (Dq, ^{b}C-3', J = 175.0, 4.2 Hz); 124.7 (D, C-5, $^1J_{CH}$ = 192.7 Hz); 88.5 (Dtq, C-4', J = 148.3, 10.0, 2.4 Hz); 84.5 (Dt, C-1', J = 153.8, 10.7 Hz); 64.4 (Tdd, C-5', J = 141, ca. 3, ca. 7 Hz); [a] Two $^3J_{CH}$ to H-5, H-2'; 1.7 Hz coupling to H-1' or H-3'.

TLC: R_f 0.7. Anal. Calcd. for $C_9H_{10}N_2O_3S$: C, 47.77; H, 4.45; N, 12.38; S, 14.17. Found: C, 47.80; H, 4.62; N, 13.13; S, 13.94.

2-(2,3-Dideoxy-ß-D-glycero-pentofuranosyl)thiazole-4-carboxamide (11) (2',3'-dideoxytiazofurin): Tiazofurin-2'-ene (2.5 g, 10 mmol) was dissolved in methanol (100 mL), and maintained under a nitrogen atmosphere. Carefully 5% ethanol-pretreatment palladium on barium carbonate (1 g) was introduced, and the hydrogenation was carried out at room temperature and atmospheric pressure during a two hour period. The catalyst was filtered off, the solvent was evaporated under reduced pressure and the residue was recrystallized from ethyl acetate; yield 2.1 g (84%); m.p. 94-95°. Analysis showed that the compound crystallized with 0.5 mol of water. IR (KBr): 3400-3050; 1670; 1380; 1190; 1050; 940 cm^{-1}.

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[2]Definitive assignment from ^{13}C spectrum based on hmbc present for H-5'a,b: δ 6.17, but not for δ 6.13.

[3]Assigned from hmbc.

^{1}H NMR: (DMSO d_6) δ 8.81 (s, 1, C_5H); 7.79 and 7.59 (each bs, 1 each, NH, partially exchanged with D_2O); 5.20 (dd, 1, H-1', J(1'-2'a,b) = 5.4, 7.9 Hz)[a4]; 4.88 (t, 1, 5'-OH, partially exchanged with D_2O, J = 5.5 Hz); 4.08 (tdd, 1, H-4', J(4'-5'a,b) = 5.3 Hz, J(4'-3'a,b) = 6.1, 7.5 Hz); 3.54 and 3.49[a] (each dd, 1 each, H-5'a,b, J(5'a,b-4') = 5.4, 5.2 Hz, J(gem) = 11.3 Hz); 2.41 (cm, 1, H-2'a); 2.03 (cm, 2, H-2'b, H-3'b); 1.71 (cm, 1, H-3'a).

[a5]Irradiation of H-4' produced no change in the absorption of H-1', indicating the absence of H(1'-4') coupling through the ribosyl oxygen or through C_2-C_3. The latter was observed when C_2-C_3 was unsaturated. Irradiation of H-4' gave rise to an AB pattern for H-5'a,b.

^{13}C-NMR: (DMSO d_6) 175.30 (Std, C-2, J = 4.5, 7.2 Hz); 162.80 (S, C-4); 150.15 (Sddd, C=O, J = 0.8, 4.9, 6.8 Hz[b6]); 124.38 (D, C-5, J_{CH} = 192.3 Hz); 81.73 (Dcm, C-4', J = 147.4, 8.2 Hz); 78.12 (Dcm, C-1', J = 151.8, 7.3 Hz); 63.93 (Tdd, C-5', J = 139.6, 2.0, 4.5 Hz); 33.09 (Tcm, C-3', J = 133.4 Hz); 27.71 (Tcm, C-2', J = 129.2 Hz);

TLC: Rf 0.70. Anal. Calcd. for $C_9H_{12}N_2O_3S$.: C, 47.35; H, 5.30; N, 12.27; S, 14.04. Found: C, 47.16; H, 5.41; N, 12.13; S, 13.78.

2-(5-Hydroxymethylfuran-2'-yl)thiazole-4-carboxamide (12):

Tiazofurin (8) (2.6 g, 10 mmol) was suspended in acetonitrile (30 mL) containing water (0.18 mL, 10 mmol) and α-acetoxyisobutyryl bromide (2) (4.5 mL, 30 mmol) is added in one portion. The reaction mixture was stirred for two hours when it formed a clear solution. Ethyl acetate (200 mL) was added, and the solution was washed with 5% sodium bicarbonate solution (2 x 50 mL), the aqueous phase was extracted with ethyl acetate (100 mL x 2), and the combined organic extracts were washed with water (50 mL) and brine (50 mL). After drying over

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[4]In a D_2O-exchanged sample, J=0.9; 4.6 Hz.

[5]Definitive assignment from ^{13}C spectrum based on hmbc present for H-5'a,b: δ 6.17, but not for δ 6.13.

[6]In a D_2O-exchanged sample, J=0.9; 4.6 Hz.

sodium sulfate the solvent was evaporated to yield a crude reaction product mixture (9).

The crude product was dissolved in anhydrous methanol (100 mL) and sodium methoxide (1.5 g) was added to adjust the pH value to 10. After stirring for two hours at room temperature the reaction mixture was neutralized with Amberlite resin H^+ (20 g). The resin was collected by filtration, the solvent was evaporated and the residue was recrystallized from methanol (25 mL) to yield 1.9 g (85%) of pure product, m.p. 192-194°; (lit. 192-193°). IR (KBr): 3420; 3380-3050(br); 1680; 1550; 1380; 1295; 1070; 1020; 890; 810 cm^{-1}.

^{1}H NMR: (DMSO d_6) δ 200 MHz 8.25 (s, 1, C_5H); 7.75 and 7.66 (each bs, 1 each, exchangeable with D_2O, NH); 7.11 (d, 1, ^{a}H-2', J(2'-3') = 3.4 Hz); 6.55 (d, 1, ^{a}H-3', J(3'-2') = 3.4 Hz); 5.45 (t, 1, 5'-OH, exchangeable with D_2O, J = 5.6 Hz); 4.50 (d, 2, H-5'a,b, J= 5.35 Hz); [a7]

^{13}C-NMR: (DMSO d_6) δ (Coupled with D_2O exchange); 162.35 (Sd, C-4, ^{2}JCCH = 1.4 Hz); 157.8 (Scm,[b8] C-4'); 157.2 (Sd, C-1', ^{2}JCCH = 7.7 Hz); 151.0 (Sdd, C=O, ^{3}JCCCH = 4.6 Hz, ^{2}JCNH = 7.2 Hz); 147.0 (Sdd, C-2'[b] ^{3}JCSCJ = ^{3}JCCCH = 8.4 Hz); 123.5 (Ds, C-5'[b] $^1J_{ch}$ = 194.7 Hz[c])[9]; 110.8 (Dd, C-2', J = 178.3, 4.6 Hz); 109.8 (Ddt, C-3', J = 177.3, 2.8, 3.8 Hz); 55.7 (Td, C-5', J = 142.8, 2.9 Hz).

TLC: Rf 0.55. Anal. Calcd. for $C_9H_8N_2O_3S$: C, 48.20; H, 3.60; N, 12.50; S, 14.30. Found: C, 48.38; H, 3.72; N, 12.49; S, 14.16.

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[7]Tentatively assigned. Similar chemical shifts and couplings were reported by Srivastava, et al[5]

[8]Most highly coupled carbon.

[9]The carbon chemical shifted and assignments for the thiazole ring for compounds 10-12 (Scheme 2) agree generally with those of Kovacs, et al. (23). The sole exception was C-2 of 12 which was shifted upfield to 147 ppm from its usual absorption at 172-3 ppm by the direct bonding to the furanosyl ring.

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